MERCK MILLIPORE

Neuroscience

Solutions for productive research

Merck Millipore is a business of MERCK

Introduction

Neuroscience is a heterogeneous field, requiring integrated analysis of multiple cell types, tissues and organs, using diverse techniques. Given that researchers tend to build upon traditional technologies rather than abandon them, the next advance in neuroscience will likely rely on antibodies and immunodetection.

Antibody-based biomarker detection reagents are now available for numerous cell and tissue types, disease states, and for measuring responses to targeted therapies. These extremely specific, robust immunoprobes are now used with high-tech fluorescent secondary antibodies and powerful microscopes, keeping alive the classic technique of immunocytochemistry in neuroscience research. Western blotting, another established immunodetection technique for cell and tissue lysates, has been vastly improved by membrane (and probe) technology.

Multiparametric biomarker detection also combines old and new technologies. Advances based on the classic enzyme-linked immunosorbent assay (ELISA) for biomarker quantification have yielded multiplex assays that exploit antibody-fluorophore conjugates for efficient, precise measurement of nearly 100 analytes per sample. Even the technique of flow cytometry, historically limited to users with specialized expertise, has vastly broadened with the availability of precision antibody-based reagents for biomarker detection. New, inexpensive systems and good neuro-specific biomarkers have granted flow cytometry access to neuroscientists in cancer research and regenerative medicine.

Recognizing the opportunities, as well as the challenges facing scientific research, Merck Millipore has been dedicated to developing and refining tools and technologies for the study of neuroscience. With Merck Millipore's comprehensive portfolio, including the Upstate®, Chemicon®, and Calbiochem® brands of reagents and antibodies, researchers can count on dependable, high quality solutions for investigating topics related to neuroscience.

In this guide, you will find key topics and latest findings related to neuroscience research, and also discover Merck Millipore's solutions for the investigation of the processes associated with the various topics. In addition, you will learn about advances in technology and technical tips. With so many effective solutions, it is our hope that you will take your neuroscience-related life science research to the next level with Merck Millipore as your partner.

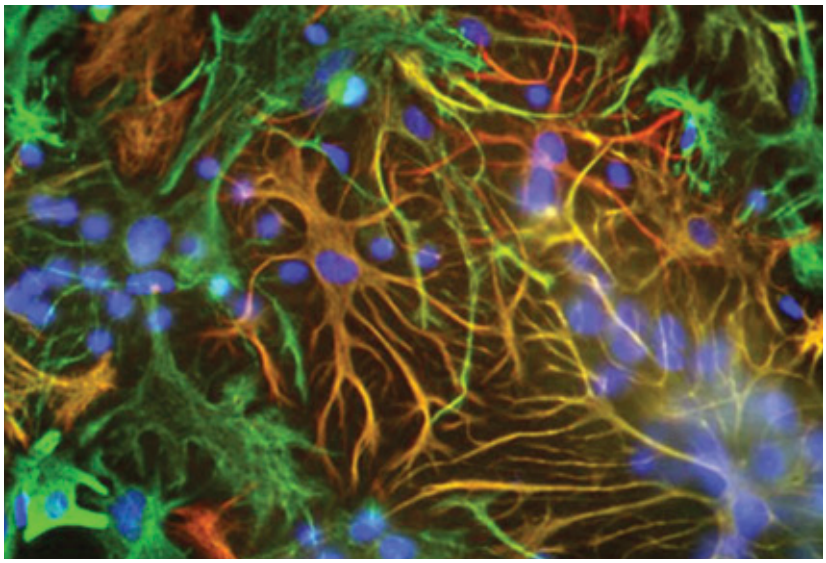
Sincerely,
Scientists like you!
Merck Millipore

Table of Contents

Synapses, Neurons, and Glia

The nervous system is composed of an estimated 100 billion neurons and at least that many glial support cells with an astounding diversity of subtypes.

Part of the excitement in modern synaptic research is in understanding the diversity of interactions at both the cellular and molecular levels. New research efforts have shown that interactions in the synaptic proteome, or synaptome are much more complex than previously thought, and that there is potentially huge regional, gender, age, and disease related influence on neural and glial cell subtypes. The challenge for researchers is to identify structure/function differences in synapses, despite not having overt anatomical markers. Merck Millipore works extensively with neuroscientists to create key neural/glial subtype marker antibodies, and is a leading supplier of well-published antibodies for synaptic proteins, and cell culture reagents.



Staining of a mixed population of neural cells from rat cerebral cortex using anti-Vimentin (Catalog No. AB5733, green) and anit-GFAP (Catalog No. AB5408, red). Nuclei are stained blue. Photo courtesy of EnCor Biotechnology, Inc.

Did You Know?

Neural synaptic connections require extreme specialization on both pre- and post-synaptic regions. Since communication occurs in both directions, it is probably more accurate to say "neurons synapse with" a target, not "neurons synapse on".

New directions in synaptic and glial research

Neural astrocyte circuits: Martin R, Bajo-Grañeras R, Moratalla R, Perea G, Araque A. Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. Science. 2015; 349(6249):730-734.

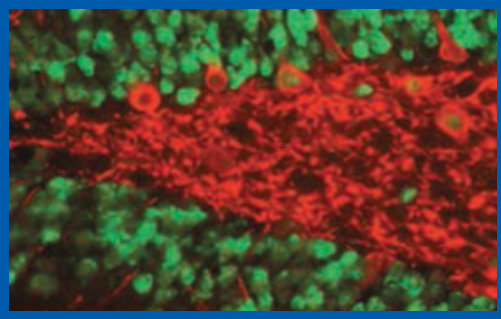
Targeting disease using the Synaptome: Tang B, Wang T, Wan H, Han L, Qin X, Zhang Y et al. Fmr1 deficiency promotes age-dependent alterations in the cortical synaptic proteome. PNAS. 2015; 112(34):E4697-E4706.

Gender differences in molecular signaling: Tabatadze N, Huang G, May RM, Jain A, Woolley CS. Sex differences in molecular signaling at inhibitory synapses in the hippocampus. J Neurosci. 2015; 35(32):11252-11265.

ANTIBODY SPOTLIGHT

Neuronal Nuclei Marker: Anti-NeuN

Our exclusive NeuN antibody (clone A60) specifically recognizes the DNA-binding, neuron-specific protein NeuN, which is present in most CNS and PNS neuronal cell types of all vertebrates tested. NeuN is apparently restricted to neuronal nuclei, perikarya, and some proximal neuronal processes in both fetal and adult brain. NeuN antibodies are routinely used in neuroscience laboratories and are cited in thousands of publications to date. Anti-NeuN is now available as a direct conjugate and also in a rabbit polyclonal format.



Mouse anti-NeuN (MAB377). Immunolocalization of NeuN (red) and BrdU (green) in the neurogenic regions of the mouse brain (dentate gyrus and subventricular zone). Photo courtesy of J.G. Emsley and T Hagg.

Advances in Technology: Synaptic imaging with Array Tomography

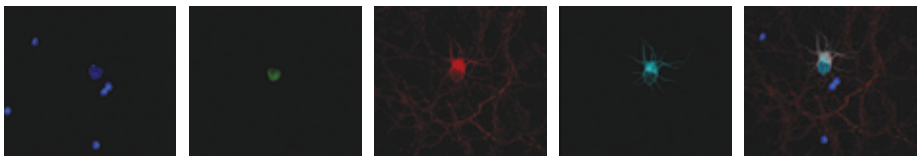
Immunohistochemical multiplexing capability lets you visualize multiple (dozen) synaptic molecules together. Stephen J Smith's lab has pioneered a technique to take the ultrathin serial section ribbon arrays from a fixed brain, stain them multiple times by removing the antibodies using a high-pH elution solution, and reconstructing the brain in 3D. This ATomo technique allows the resolution of individual synapses *in situ* within brain tissue specimens. The throughput of the technique is inherently high, approaching the imaging of one million synapses per hour. Smith discussed with Merck Millipore scientists that the technique requires the use of validated, high precision antibodies with minimal cross-reactivity.

Collman F, Buchanan J, Phend KD, Micheva KD, Weinberg RJ, Smith SJ. J Neurosci. 2015; 35:5792-5807.

Featured Technique:

Whole Cell Visualization

Visualization of the complete cellular architecture is often necessary to discern cell types or structure/function relationships. While Golgi staining and fluorescent (GFP/YFP) constructs yield excellent cytoarchitectural detail, these approaches are technically challenging and impractical. Antibody staining of key neuronal proteins is effective for identifying neurons and discerning regional morphological characteristics, such as nuclei (NeuN), post-synaptic membrane (PSD-95), or spines (spinophilin). Although neuron-specific antibodies reveal cytoarchitecture, they are limited by the protein distribution within the neuron, and it becomes necessary to use multiple antibodies (e.g., triple staining) to different regional markers to get whole cell visualization.



DAPI | Nuclear Alexa Fluor® 488 dye | Somato-axonal Cy3 | Somato-dendric Alexa Fluor® 647 dye | Merge

ChromaPan Neuronal Marker antibody blend (Catalog No. NS420) was tested on rat cortex primary neurons (E18). Image was taken using a confocal microscope with appropriate filters. Fluorophore Alexa® 647 is represented in pseudo color.

Technical Tip

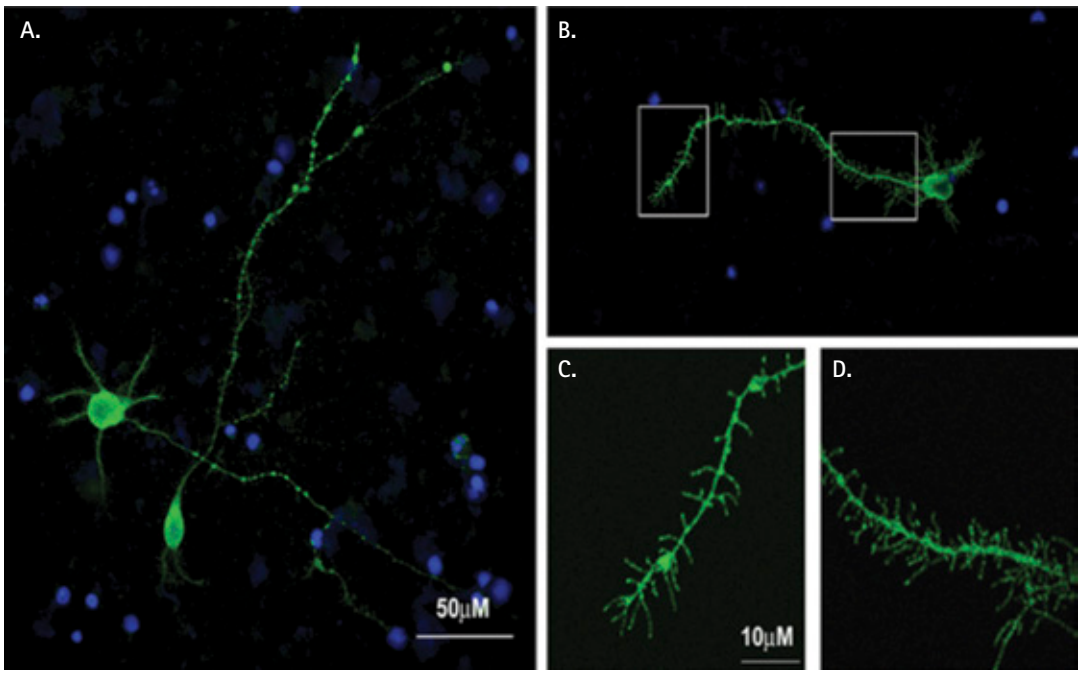
Identifying neural subtypes does not necessarily require the difficult task of targeting a particular neurotransmitter (e.g. GABA). There are often many other protein targets related to particular neurotransmitter production and storage that make excellent neural or glial subtype specific markers (e.g. vGAT).

Featured Solution:

FluoroPan Neuronal Marker

(Catalog No. MAB2300X)

To achieve complete staining across all parts of neurons, Merck Millipore has developed a family of pan-neuronal antibody blends that label key somatic, nuclear, dendritic, spine, and axonal proteins. These pan-neuronal antibody cocktails have been validated in a variety of cell and tissue cultures, and offer researchers a convenient, specific, qualitative, and quantitative system for studying neuronal morphology. One example of this class of unique pan neuronal markers is the FluoroPan neuronal marker, an Alexa Fluor® 488 dye-conjugated monoclonal antibody blend, developed to enable detection of an entire neuron with only one microscopic emission channel. Other unconjugated or conjugated monochromatic and three color blends are also available.



High morphological resolution has been achieved using MAB2300X, with clear staining of dendrites, soma, nucleus and axon (A and B). A higher magnification image of image B inserts showing spines (C and D).

Also Available:

Description	Use	Catalog No.
Pan Neuronal Marker – Unconjugated	Reseachers can choose secondary antibody & can co-label using any non-mouse hosted antibodies.	MAB2300
FluoroPan Neuronal Marker – Alexa® 488 dye-conjugated	No secondary antibody needed! Researchers can co-label using any host.	MAB2300X
ChromaPan Neuronal Marker	Neuron parts stained in different colors. Primary antibody and secondary antibody blend makes simple protocol.	NS420
ChromaPan Neuronal Marker – Ms open (OMC)	No mouse host is used so researchers can co-label using their own mouse antibodies for the Alexa Fluor® 488 channel	NS330
ChromaPan Neuronal Marker – Rb open (ORC)	No rabbit host is used so researchers can use their own rabbit antibodies for the Cy3 channel.	NS340

Solutions for your Research

Merck Millipore offers effective solutions for research on Synapses, Neurons, and Glia:

Research Solutions	Description	Catalog No.
Neuronal Nuclei Marker	Anti-NeuN (guinea pig)	ABN90
	Anti-NeuN (chicken)	ABN91
	Rb Anti-NeuN polyclonal	ABN78
	Rb Anti-NeuN – Biotin Conjugated	ABN78B
	Rb Anti-NeuN – Alexa®488 Conjugated	ABN78A4
	Rb Anti-NeuN - Cy®3 Conjugated	ABN78C3
	Ms Anti-NeuN monoclonal, clone A60	MAB377
	Ms Anti-NeuN - Biotin Conjugated	MAB377B
	Ms Anti-NeuN – Alexa®488 Conjugated	MAB377X
Pan Neuronal Marker	Pan Neuronal Marker – Unconjugated	MAB2300
	FluoroPan Neuronal Marker – Alexa® 488 dye-conjugated	MAB2300X
	ChromaPan Neuronal Marker	NS420
	ChromaPan Neuronal Marker – Ms open (OMC)	NS330
	ChromaPan Neuronal Marker – Rb open (ORC)	NS340
Related Antibodies	Anti-Pan-Neuronal Neurofilament marker, clone SMI-311	NE1017-100UL
	Anti-Neurofilament M (145 kDa), C-terminus	AB1987
	Anti-Synaptophysin, clone SY38	MAB5258
	Anti-PSD-95	AB9708
Related Small Molecules	Neuronal Differentiation Inducer IV	480746
	Neurogenesis Enhancer, P7C3A20	480744
Synapse and Structure Assays	AXIS® Axon Isolation Device, Tissue Culture Ready, 150 µm	AX15010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 450 µm	AX45005TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 450 µm	AX45010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 900 µm	AX90010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 6-well	AX50010TC

For a full selection and latest releases, visit: www.merckmillipore.com

TECHNOLOGY HIGHLIGHT

Antibodies and Conjugated Antibodies

Antibodies are critical tools for most areas of life science research, primarily for their use as molecular tags for specific labeling and detection. Researchers depend on precise, dependable antibodies to study targets and discover new molecules.

Merck Millipore is a leading antibody developer providing high quality reagents validated for use in immunostaining, blotting, purification, ChIP, flow cytometry, and multiplexed assays.

Antibody Development, Validation and Guarantee

At Merck Millipore, our scientists evaluate potential antibody targets to determine if our customers can benefit from antibodies for them. We then work to produce specific, validated antibodies for success in various research applications. Our commitment to produce quality antibodies is based on innovation, customer beta-testing and their feedback to the design/engineering team. These efforts and collaborations have led to new validation techniques and novel antibody-based technologies, such as improved bead-based multiplex assays and imaging flow cytometry. Rest assured, every antibody we ship is backed by a full satisfaction guarantee.

Making the Impossible Antibody Possible

The diverse development expertise at Merck Millipore gives us higher success rate at producing precision antibodies to difficult targets. We have developed an extensive collection of state-dependent antibodies that detect phosphorylation, methylation, acetylation, etc. In addition, we have numerous conformation-specific antibodies for detecting subunit binding, or 3-dimensional structure in protein aggregation, which is very relevant to neurodegenerative and cancer research.

The Tough Targets

- Post-translational modification (PTM) antibodies
- Oligomeric or aggregate-specific antibodies
- Native 3D native conformation antibodies
- Native extracellular signaling domain structure antibodies
- Multi-application antibodies including ChIP and flow cytometry
- Multiplexed immunoassay antibodies (MILLIPLEX® bead-based assays using Luminex xMAP® technology)

At your Service

Excellent service and support, as well as popular tools, such as technical guides, training materials, pathway posters, and research reviews, enable your research. Count on us for reliable primary and secondary antibodies you need to advance your research.

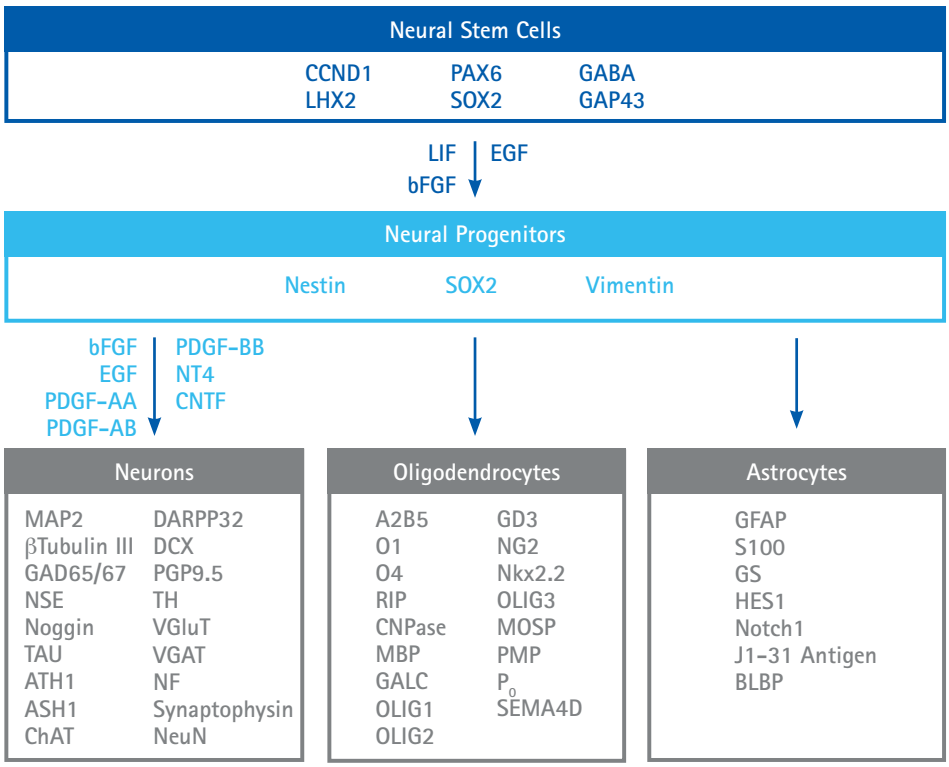
For details, visit: **www.merckmillipore.com/antibodies**

Notes

Neural Stem Cell Markers

The overly hopeful, simplistic view that neural stem cell (NSC) development can be easily manipulated for regenerative therapeutic applications has given way to a much richer understanding of the complexities involved in differentiation.

New research exploring the intrinsic and extrinsic control mechanisms of neural stem cell differentiation is redefining how we think of cell fate. In addition, new mechanisms involving extracellular vesicle (EV) signaling and miRNA/epigenetic control are proving to play important roles in both normal neural development and pathogenesis of diseases. Merck Millipore is dedicated to developing relevant human and rodent neural stem cell systems including novel cell lines derived from both adult and embryonic neural tissue, optimized media for cell expansion and differentiation, and a complete selection of lineage-specific and stage-specific antibodies.



Did You Know?

Researchers recently succeeded in mimicking Pax6-related human brain development in mouse cortical progenitor cells. Consequently, the mouse progenitors generated more neurons—a prerequisite for a bigger brain.

Wong, FK et al. (2015) PLOS 13(8) e1002217

Neural Stem Cells Markers.
Explore Validated Antibodies to the most popular NSC markers at:
www.merckmillipore.com

New directions in neural stem cell research

Intrinsic and extrinsic mechanisms regulating NSCs: Fuentealba LC, Rompani SB, Parraguez JI, Obernier K, Romero R, Cepko CL, Alvarez-Buylla A. Embryonic Origin of Postnatal Neural Stem Cells. Cell. 2015; 161(7):1644-1655.

miRNAs and exosomes in brain tumors: Godlewski J, Kricheysky AM, Johnson MD, Chiocca EA, Bronisz A. Belonging to a network-microRNAs, extracellular vesicles, and the glioblastoma microenvironment. Neuro Oncol. 2015; 17(5):652-662.

Epigenetic codes in neurogenesis: Yao B, Jin P. Unlocking epigenetic codes in neurogenesis. Genes & Dev. 2014; 28:1253-1271.

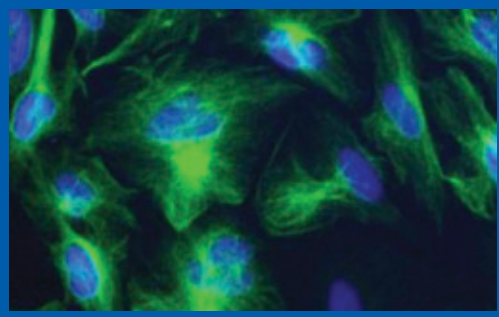
ANTIBODY SPOTLIGHT

Anti-Nestin Antibody

Nestin is a large intermediate filament protein (class Type VI) expressed during development and in myotendinous and neuromuscular junctions. Nestin expression is restricted and typically disappears by E18.

This Anti-Nestin antibody lets you specifically detect Nestin, and is recommended for use in IC, IH, IH(P) & WB. This antibody detects a ~220 kDa protein (may vary slightly depending on cell lines used).

See Solutions table at the end of this section for other Nestin-specific antibodies.

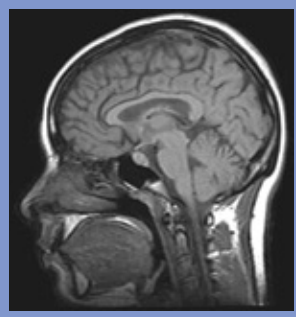


U251 cells stained with Anti-Nestin (Catalog No. AB5922, green) and bisbenzimide (blue). Photo courtesy of Dr. Conrad Messam, Dr. Jean Hou, and Dr. Eugene Major, NINDS, NIH, Bethesda, MD. Reprinted from Experimental Neurology (2000) 161:585-596.

Advances in Technology: Using MRI probes and sensors for *in vivo* cell tracking in transplantations

The ability to assess whether transplanted stem cells integrate functionally into the targeted environment continues to be a significant challenge in regenerative medicine. New advances in magnetic resonance imaging (MRI) based probes allow much improved cellular and temporal resolution. MRI's unique ability to penetrate and image deep into tissues makes it an ideal tool for tracking transplanted cell populations and evaluating delivery mechanisms.

Srivastava AK, Kadayakkara DK, Bar-Shir A, Gilad AA, McMahon MT, Bulte JWM. 2015; 8(8):323-336.



Technical Tip

Be aware of your colony's metabolic health prior to reaching the appropriate confluence percentage. Cell populations can be undergoing oxidative stress, autophagy, epigenetic changes, and early apoptosis long before any pH dye indicators change color. Such covert health changes could significantly impact neural stem growth or transformation variability.

Learn more at: www.merckmillipore/muse

Featured Technique:

Stem Cell Characterization

Neural stem cells (NSCs) are present in both the developing and adult nervous system of all mammals, including humans. They possess the remarkable capacity to self-renew and to differentiate along specific pathways to generate the vast array of neuronal and glial cell types of the central nervous system (CNS). Due to their therapeutic promise, considerable attention has been focused on identifying the sources of stem cells, the signals that regulate their proliferation and the specification of neural stem cells towards more differentiated cell lineages.

Neural stem cells are often identified based upon the presence of molecular markers that are correlated with the stem and/or progenitor state along with the absence of a more differentiated phenotype as assessed through marker analysis. Antibody based cell characterization kits are excellent tools to investigate NSC fates.

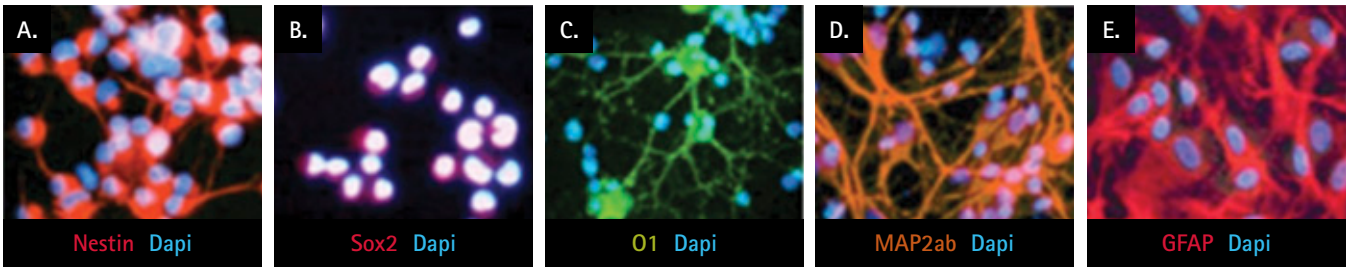
Featured Solution:

Neural Stem Cell Marker Characterization Kit

(Catalog No. SCR019)

Merck Millipore's Neural Stem Cell Marker Characterization Kit (Catalog Number SCR019) aids researchers in the accurate identification of neural stem cells. This kit contains two molecular markers: Nestin and Sox 2, that are frequently used to identify neural stem/progenitor cells along with more differentiated lineage markers including MAP2ab for neurons, GFAP for astrocytes and O1 for oligodendrocytes. Mouse and rabbit immunoglobulins for the assessment of background staining are also included.

All of the antibodies provided in the kit have been tested and optimized for use in immunocytochemistry on adult rat neural stem cells. We recommend that this kit be used in conjunction with differentiation assays that demonstrate multipotentiality of the starting cell population.



Cultured adult rat hippocampus-derived neural stem cells stained for (A) Nestin (red) and (B) Sox-2 (red). The Sox-2 transcription factor is colocalized with the DAPI (blue) staining in the nucleus. C. Mouse anti-Oligodendrocyte O1 (green) staining of adult rat hippocampus-derived neural stem cells that have been exposed to differentiation conditions for four days. D. Localization of MAP-2 (orange) in primary rat hippocampus-derived embryonic neurons (Catalog No. SCR009, SCR010) that have been thawed and cultured for ten days. E. Primary rat hippocampus-derived embryonic astrocytes (Catalog Nos. SCR007, SCR008) stained for GFAP (red). Nuclei of the cells were visualized with DAPI (blue).

Other Stem Cell Characterization Kits:

Description	Catalog No.
ES Cell Characterization Kit	SCR001
Adult Rat Hippocampal Neural Stem Cell Kit	SCR021
Human Embryonic Germ Layer Characterization Kit	SCR030
Human Neural Stem Cell Characterization Kit	SCR060
Quantitative Alkaline Phosphatase ES Characterization Kit	SCR066
Fluorescent Mouse ES/iPS Cell Characterization Kit	SCR077
Fluorescent Human ES/iPS Cell Characterization Kit	SCR078
Human Oligodendrocyte Characterization Kit	SCR601
Embryonic Stem Cell Derived Neuron Integration and Characterization Kit	NS140
FlowCellect® Human ESC Nucleus Marker Characterization Kit	FCHEC25102
FlowCellect® Human ESC (HESCA-1) Surface Marker Characterization Kit	FCHEC25104
FlowCellect® Human ESC (TRA-1-60) Surface Marker Characterization Kit	FCHEC25106
FlowCellect® Mouse ESC Nuclear Marker Characterization Kit	FCMEC25110
FlowCellect® Rodent NSC Characterization Kit (Neural)	FCRNC25112
FlowCellect® Human iPS Cell Characterization Kit	FCSC100107

Solutions for your Research

Merck Millipore offers effective solutions for research on Neural Stem Cells:

Research Solutions	Description	Catalog No.
Neuronal Stem Cell Marker Detection	Anti-BCRP, clone BXP-21	MAB4146
	Anti-CD133, clone 13A4	MAB4310
	Anti-Mouse Nanog, N-Terminus	ABD88
	Anti-Musashi-1	AB5977
	Anti-Nanog	AB9220
	Anti-NANOG, clone 7F7.1, Alexa Fluor® 488 conjugate	MABD24A4
	Anti-NANOG, clone 7F7.1, Cy®3 conjugate	MABD24C3
	Anti-Nanog, N-terminus	AB5731
	Anti-Nestin	ABD69
	Anti-Nestin, clone 10C2	MAB5326
	Anti-Nestin, clone 10C2, Alexa Fluor® 488 conjugate	MAB5326A4
	Anti-Nestin, clone 10C2, Cy®3 conjugate	MAB5326C3
	Anti-Nestin, clone rat-401	MAB353
	Anti-Nestin, clone rat-401, Alexa Fluor® 488 conjugate	MAB353A4
	Anti-Nestin, clone rat-401, Biotin Conjugate	MAB353B
	Anti-Nestin, clone rat-401, Cy®3 conjugate	MAB353C3
	Anti-Oct-4	ABE422
	Anti-OCT-4 [POU5F1], clone 7F9.2	MAB4419
	Anti-OCT-4 [POU5F1], clone 7F9.2, Alexa Fluor® 488 conjugate	MAB4419A4
	Anti-OCT-4 [POU5F1], clone 7F9.2, Cy®3 conjugate	MAB4419C3
	Anti-Oct-4, clone 10H11.2	MAB4401
	Anti-Oct-4, clone 10H11.2, Alexa Fluor® 488 conjugate	MAB4401A4
	Anti-Oct-4, clone 10H11.2, Alexa Fluor® 488 conjugate	FCMAB113A4
	Anti-Oct-4, clone 10H11.2, Cy®3 conjugate	MAB4401C3
	Anti-Oct-4, clone 9B7	MABD76
	Anti-Oct-4, clone 9E3.2	MAB4305
	Anti-SnoN	ABD104
	Anti-SOX-15, clone 1A2.1	MABD94
	Anti-SOX-2	MAB4343
	Anti-SOX-2 , clone 10H9.1, Cy®3 conjugate	MAB4423C3
	Anti-SOX-2 , clone 6G1.2, FITC conjugate	FCMAB112F
	Anti-SOX-2, clone 10H9.1	MAB4423
	Anti-SOX-2, clone 10H9.1, Alexa Fluor® 488 conjugate	MAB4423A4

Continued on next page

Merck Millipore offers effective solutions for research on Neural Stem Cell Markers:

Research Solutions	Description	Catalog No.
Neuronal Stem Cell Marker Detection	Anti-Sox2	AB5603
	Anti-Sox2 Mouse mAb (245610)	SC1002
	Anti-Sox2, Alexa Fluor® 488 Conjugate	AB5603A4
	Milli-Mark® Anti-Nestin-PE, clone 10C2	FCMAB313PE
	Milli-Mark® Anti-mOCT4, clone 7F9.2, Alexa Fluor® 488 Conjugate	FCMAB124A4
Neural Stem Cells, Kits & Assays	ENStem™-A Human Neural Progenitor Expansion Kit	SCR055
	ES2N Complete Medium Kit (includes ES2N Basal Medium plus Neuro27 and Neuro2 supplements)	SCM082
	Human Neural Stem Cell Characterization Kit	SCR060
	Human Oligodendrocyte Differentiation Kit	SCR600
	MilliTrace™ Constitutive GFP Reporter Adult Rat Hippocampal Neural Stem Cell Kit	SCR080
	MilliTrace™ CX Constitutive GFP Reporter Cell Line	SCR095
	MilliTrace™ CX Nestin GFP Reporter Cell Line	SCR096
	MilliTrace™ Nanog GFP Reporter Mouse Embryonic Stem Cell Kit	SCR089
	MilliTrace™ VM Constitutive GFP Reporter Cell Line	SCR092
	Mouse Cortical Neural Stem Cells	SCR029
	Mouse Spinal Cord Neural Stem Cells	SCR031
	N21 Medium Supplement (50X)	SCM081
	Neural Stem Cell Marker Characterization Kit	SCR019
	O9-1 Mouse Cranial Neural Crest Cell Line	SCC049
	Rat Hippocampal Neural Stem Cells	SCR022
	ReNcell® CX Human Neural Stem Cell Kit	SCC009
	ReNcell® VM Human Neural Stem Cell Kit	SCC010
Small Molecule Inhibitors	DNA Methyltransferase Inhibitor II, SGI-1027	260921
	Histone Acetyltransferase p300 Inhibitor, C646	382113
	InhibitorSelect™ 384-Well Protein Kinase Inhibitor Library I	539743
	InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library II	539745
	InhibitorSelect™ 96-Well Protein Kinase Inhibitor Library III	539746
	StemSelect® Small Molecule Regulators 384-Well Library I	569744

For a complete selection, visit: www.merckmillipore.com

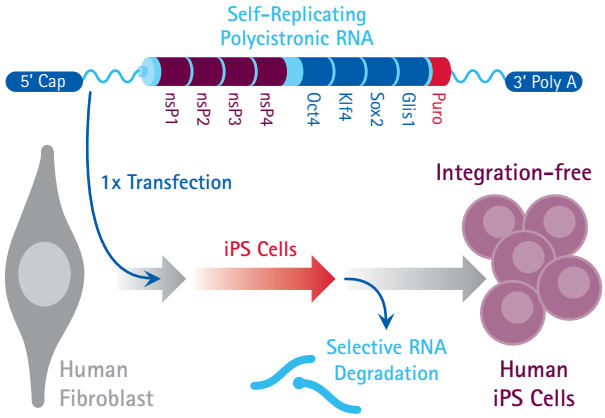
TECHNOLOGY HIGHLIGHT

Simplicon™ RNA Reprogramming Technology

Single transfection. Virus-free iPSCs.

Drawbacks of current iPSC generation methods, involving virus or multiple transfections, include experimental variability, questions of biosafety, and known and unknown effects of introducing viruses into experimental systems.

We challenge you to rethink your reprogramming strategy. The Simplicon™ RNA Reprogramming Technology utilizes a single, polycistronic, synthetic, self-replicating RNA strand that is sufficient for generating high numbers of human iPSCs with a single transfection step1. The single RNA contains the four reprogramming factors, OCT-4, KLF-4, SOX-2 and GLIS1, and allows for efficient reprogramming without viral intermediates or host genome integration. The efficiency of this new technology has been shown to range from 0.3% to 1.1%, depending on fibroblast proliferation rate.



How Simplicon™ Technology works.

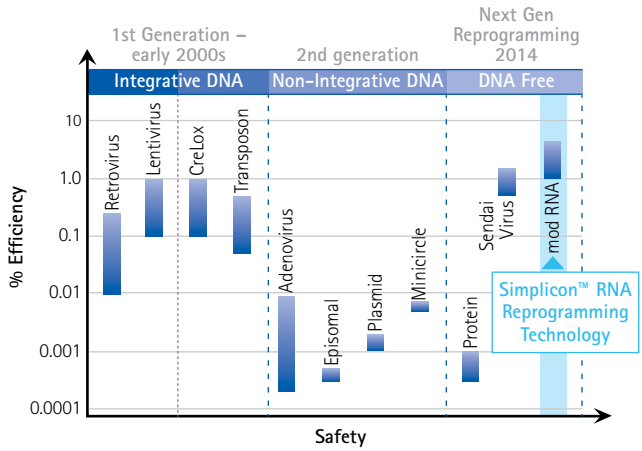
With Simplicon™ RNA Reprogramming Technology, you can:

- Create integration-free iPS cells using synthetic RNA
- Reprogram efficiently and easily using a single transfection step
- Achieve controlled elimination of reprogramming factors

References

1. Yoshioka N et al. Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell. 2013 Aug 1;13(2):246-54.
2. Bernal JA. RNA-based tools for nuclear reprogramming and lineage-conversion: towards clinical applications. J Cardiovasc Transl Res. 2013 Dec;6(6):956-68.

The Evolution of Reprogramming Technologies



Safest and most efficient. The evolution of reprogramming technologies has culminated in the development of synthetic RNA-mediated reprogramming (extreme right), representing the safest and most efficient method for iPS cell generation[2]. *Illustration adapted from Juan A. Bernal, J. of Cardiovasc. Trans. Res. (2013) 6:956–968, July 2013*

For more information, visit: www.merckmillipore.com/simplicon

Culture media to efficiently differentiate human iPS cells to neural progenitors and neurons.

Go from iPS cells to Neural Progenitors to Differentiated Neurons and Oligodendrocytes...Efficiently!

Merck Millipore has developed efficient media for your stem cell workflow. Our Neural Induction Medium (NIM, Catalog No. SCM110) and Neural Differentiation Medium (NDM, Catalog No. SCM111) allow you to generate highly expandable and multipotent neural progenitors and end-stage neuronal and glial cells from induced pluripotent stem (iPS) cells. These media use established small molecule inhibitors and neural media supplements to produce rapid differentiation kinetics and efficiencies. iPS cell-derived neural progenitor cells (NPCs) can be expanded for up to 10 passages while maintaining proper growth kinetics and marker expression. These NPCs are multipotent, differentiating into neurons, astrocytes, and oligodendrocytes depending on the environmental cues introduced into the culture conditions. Specific neural subtypes, such as TH^+ dopaminergic and $VGlut^+$ glutaminergic neurons can also be generated from the NPCs using commonly used growth factor inducers. When used with Simplicon™ RNA reprogramming technology and supporting products, these media provide a complete solution for researchers looking to model neurological diseases, such as Alzheimer's disease, Parkinson's disease and autism, using an iPS cell model system.

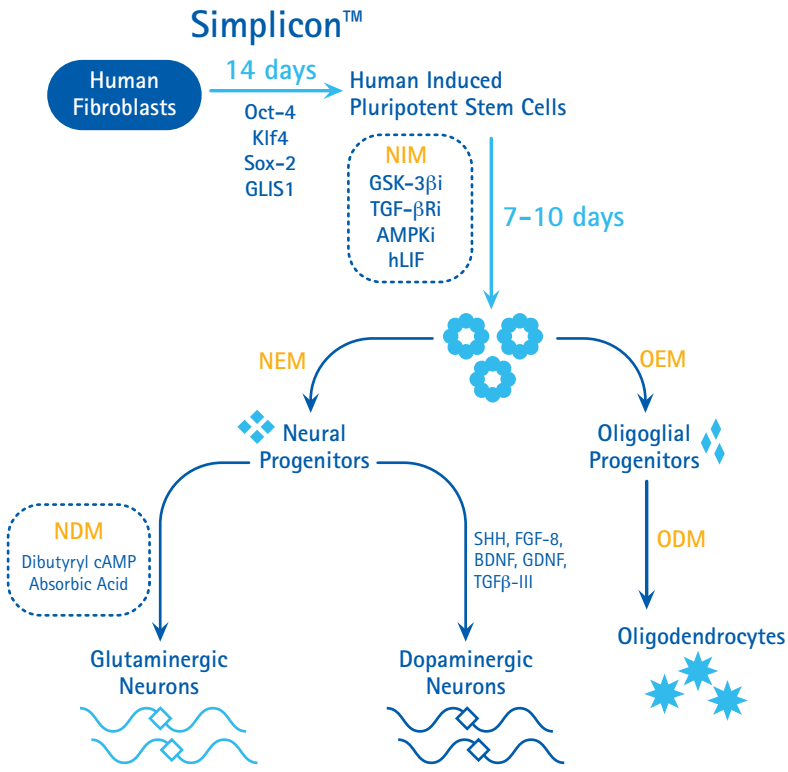
NIM: Human ES/iPS Neural Induction Medium (Catalog No. SCM110)

NEM: ENStem™-A Neural Expansion Medium (Catalog No. SCM004)

NDM: Human ES/iPS Neuronal Differentiation Medium (Catalog No. SCM111)

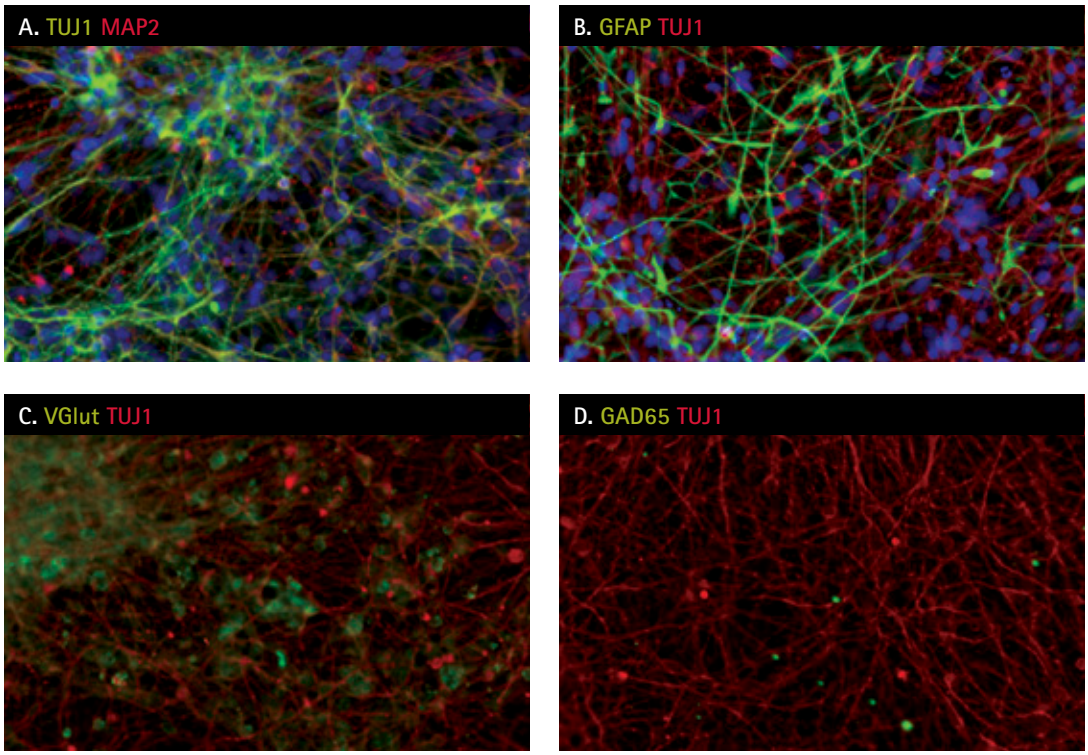
OEM: Human OPC Expansion Media Kit (Catalog No. SCM107)

ODM: Human OPC Spontaneous Differentiation Media Kit (Catalog No. SCM106)



Workflow showing all steps in iPS cell generation and subsequent differentiation to neural lineages. In as few as four steps, adult fibroblasts can be converted to neural lineages using media formulations for modulating cell fate. Along with iPS cell-generating reprogramming technologies (Simplicon™ RNA Reprogramming Kits), Merck Millipore now offers media to generate different neural and glial subtypes from iPS cells for "disease-in-a-dish" researchers.

As illustrated below, iPS cell-derived neural progenitors can be differentiated into terminally differentiated neurons using Neuronal Differentiation Medium (NDM). Over 70% of differentiated cells were positive for TUJ1 and MAP2, markers of mature neurons, while less than 20% of the differentiated cells were positive for GFAP, a marker of astrocytes. Terminally differentiated neuronal cells were preferentially glutaminergic ($VGlut^+$) rather than GABAergic neurons ($GAD65^-$) using NDM.



Neuronal differentiation of iPSC-derived NPCs. After 9 days of differentiation, cells showed extended, elaborate neurite networks. The majority of the differentiated cells expressed neuronal markers, TUJ1 and MAP2 (A) with some cells differentiating to $GFAP^+$ astrocytes. (B). Terminally differentiated cells were preferentially glutaminergic (C) rather than GABAergic neurons (D).

For more information, visit: **www.merckmillipore.com/stemcells**

Neurotrophic Factors

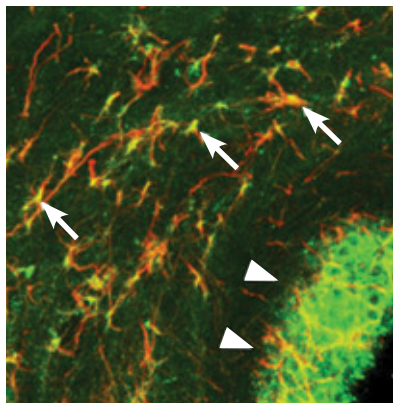
Signaling molecules that support the development and maintenance of different neuronal populations within the central and peripheral nervous systems are potential targets for development of therapeutic strategies to treat neurodegenerative diseases and peripheral neuropathies.

Brain development, including mature wiring related to memory and learning, is partly regulated by intracellular and extracellular signaling molecules. Neurotrophins mediate a wide range of neuronal functions, including survival, differentiation, growth, and death, via two classes of transmembrane receptors, the Trk receptor tyrosine kinase and the p75 neurotrophin receptor. Neurotrophic factors not only have broad ranging effects, but also are influenced by diverse intrinsic and extrinsic stimuli. Numerous recent publications have elucidated the cellular, genetic, and epigenetic effects of various pharmaceuticals on brain-derived neurotrophic factor (BDNF) levels. Neurotrophic factors have also been implicated in nerve-cancer cell cross-talk. Cancer cells of many types apparently produce nerve growth factor (NGF) to promote axon outgrowth and nerve infiltration, causing neurotransmitter release, which in turn alters the tumor microenvironment and fuels tumor growth.

Merck Millipore has worked with pioneering neurotrophin researchers to develop and supply key antibodies and ELISAs validated both internally and with publications for use in various systems.

Did You Know?

Stanley Cohen and Rita Levi-Montalcini received the 1986 Nobel Prize in Physiology for their discovery of "growth factors", specifically NGF and EGF.



Rat brain section stained with Anti-Nerve Growth Factor, pro (Catalog No. AB5583, green) reveals staining in astrocytes of the upper corpus callosum (arrows) and in periventricular cells (white triangles). Astrocytes were stained using Anti-GFAP (red).

New directions in neurotrophic factor research

Neural activation of neurotrophins: Wong Y-H, Lee C-M, Xie W, Cui B, Poo M-m. Activity-dependent BDNF release via endocytic pathways is regulated by synaptotagmin-6 and complexin. Proc. Natl. Acad. Sci. USA. 2015; 112(32):E4475-E4484.

Epigenetics of BDNF in Depression: Duclot F, Kabbaj M. Epigenetic mechanisms underlying the role of brain-derived neurotrophic factor in depression and response to antidepressants. J Exp Biol. 2015; 218:21-31.

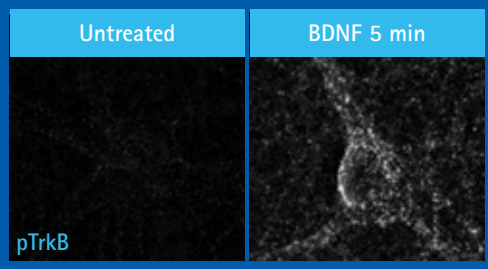
Neuronal promotion of cancer: Jobling P, Pundavela J, Oliveira SM, Walker MM, Hondermarck H. Nerve-Cancer Cell Cross-talk: A Novel Promoter of Tumor Progression. Cancer Res. 2015; 75(9):1777-1781.

ANTIBODY SPOTLIGHT

Trk Antibodies: Anti-phospho-TrkB (Tyr816)

Neurotrophins mediate a wide range of neuronal functions via transmembrane receptors, such as the Trk receptor tyrosine kinase. TrkA is selective for NGF, TrkB is selective for BDNF and NT-4, and TrkC is selective for NT-3. Merck Millipore offers a broad range of target specific antibodies for neurotrophins and their receptors.

Detect phospho-TrkB (Tyr816) using this Anti-phospho-TrkB (Tyr816) Antibody validated for use in WB, ICC, IHC, and IP.



Cultured primary rat hippocampal neurons were left untreated (left image) or subjected to 50 ng/mL BDNF stimulation for 5 minutes (right image) before staining with Anti-phospho-TrkB (Tyr816) (Catalog No. ABN1381). (Image courtesy of Dr. Moses V. Chao, Langone Medical Center, New York University.)

Advances in Technology

NGF binds to and activates its high affinity receptor (TrkA), and is internalized into the responsive neuron. The NGF/TrkA complex is subsequently trafficked back to the cell body. This movement of NGF from axon tip to soma is thought to be involved in the long-distance signaling of neurons. Researchers have been able to exploit this by using anti-p75 low affinity nerve growth factor receptor (LNGFR or p75 neurotrophin receptor) antibodies conjugated to the toxin saporin to stimulate the uptake of the receptor/toxin complex and consequently ablate the neurons. Learn more at: www.merckmillipore.com. Search "saporin".

Technical Tip

Some neurotrophic factors such as BDNF and NGF have pro-forms that may be biologically active. When choosing an antibody against neurotrophins, consider whether an N terminal or C terminal epitope will be necessary.

Featured Technique:

Chemotaxis and Migration Assays

Chemotaxis and cell migration are fundamental to normal biological processes, including embryonic development, angiogenesis and wound healing, and inflammatory response. The migration of developing neuronal cells is critical to the formation of the nervous system. Chemotaxis and migration towards neurotrophic factors have been investigated *in vitro* to understand the dynamics of their action. Many researchers studying cell migration employ the more quantitative Boyden chamber technique. Two forms of cell migration that can be studied using Boyden chambers are chemotaxis and haptotaxis.

Merck Millipore's ever-evolving portfolio of chemotaxis and migration solutions includes numerous Boyden chamber based assays, but is also moving beyond this classic technique to allow a dynamic analysis of migration, as with the Millicell® µ-Migration Assay Kit and the CellASIC® ONIX Microfluidic Platform.

Featured Solution:

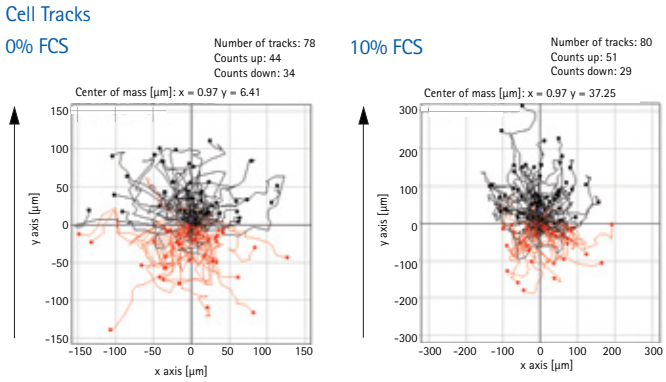
Millicell® µ-Migration Assay Kit

(Catalog No. MMA205)

Measure the Effects of Chemoattractants on the Migration of Adherent Single Cells Through Real-Time Imaging

This unique platform makes it easy to measure the effects of chemoattractants or chemorepellents on the migration of adherent single cells with real-time imaging. A concentration gradient can be established for more than 48 hours; chemotaxis can be distinguished from random movement. The glass-like properties of the slide promote enhanced optical imaging for both slow- and fast-migrating cells thus allowing for multiparametric analysis for greater mechanistic insight. Parameters such as cell velocity, directionality and index can be quantified. Data is easily analyzed using free Image J plug-in software.

You won't get this kind of information with traditional migration assay platforms such as Boyden chambers or scratch (wound healing) assays. Data was analyzed and graphed using a free Image J software plug-in.



For detailed videos and instructions on the use of the µ-migration kit, please visit: **www.millipore.com/umigration**

Also available:

If you prefer Boyden Chamber assays, Merck Millipore offers a broad selection of kits for this widely accepted cell migration technique. The classic Boyden chamber system uses a hollow plastic chamber, sealed at one end with a porous membrane. This chamber is suspended over a larger well which may contain medium and/or chemoattractants. Cells are placed inside the chamber and allowed to migrate through the pores, to the other side of the membrane. Migratory cells are then stained and counted. Merck Millipore's portfolio of chemotactic, haptotactic, migration, and invasion Boyden chamber cell-based assays enable researchers to simulate the conditions encountered by migrating cells *in vivo*. See table below for selection.

Description	Pore Size	Plate Format	ECM Coating	Detection	No. Tests	Catalog No.
Chemotaxis Cell Migration Assays	8 µm	24-well	None	Colorimetric	24	ECM508
		24-well		Fluorometric	24	ECM509
		96-well		Fluorometric	96	ECM510
	5 µm	24-well		Colorimetric	24	ECM506
		24-well		Fluorometric	24	ECM507
		96-well		Fluorometric	96	ECM512
	3µm	24-well		Colorimetric	24	ECM504
		24-well		Fluorometric	24	ECM505
		96-well		Fluorometric	96	ECM515
Haptotaxis Cell Migration Assays	8 µm	24-well	Fibronectin	Colorimetric	24	ECM580
		24-well	Vitronectin	Fluorometric	24	ECM581
		24-well	Collagen I	Fluorometric	24	ECM582
	5 µm	24-well	Laminin vials	Colorimetric	24	ECM220
		24-well		Fluorometric	24	ECM221

Solutions for your Research

Merck Millipore offers effective solutions for research on Neurotrophic Factors:

Research Solutions	Description	Catalog No.
Neurotrophic Factor and Receptor Detection	Neurotrophin Set Trial Pack 1, Contains 100 µg each of Sheep IgG Fraction anti-BDNF, NGF, NT3, NT4/5	NS110
	Anti-Brain Derived Neurotrophic Factor	AB1513
	Anti-Brain Derived Neurotrophic Factor	AB1779SP
	Anti-Brain Derived Neurotrophic Factor	AB1779
	Anti-Brain Derived Neurotrophic Factor	AB1513P
	Anti-Brain Derived Neurotrophic Factor	AB1534SP
	Anti-Brain Derived Neurotrophic Factor	AB5555P
	Anti-Brain Derived Neurotrophic Factor	AB1534
	Anti-Brain Derived Neurotrophic Factor, pro	AB9042
	Anti-Brain Derived Neurotrophic Factor, pro	AB5613
	Anti-Brain Derived Neurotrophic Factor, pro	AB5613P
	Anti-Nerve Growth Factor	AB927
	Anti-Nerve Growth Factor, clone 27/21	AB5260-60UG
	Anti-Nerve Growth Factor, clone 27/21, azide free	MAB5260Z
	Anti-Nerve Growth Factor, clone N60	MAB5744
	Anti-Nerve Growth Factor, pro	AB5583-50UL
	Anti-Nerve Growth Factor Receptor (p75), clone 8211	MAB5264
	Anti-Nerve Growth Factor Receptor (p75), clone ME20.4	MAB5386
	Anti-Nerve Growth Factor Receptor, Alexa Fluor® 488 conjugated	MAB5592X
	Anti-Nerve Growth Factor Receptor, clone MLR2	MAB5592
	Anti-Nerve Growth Factor Receptor, extracellular, clone 192-IgG	MAB365
	Anti-Nerve Growth Factor Receptor, p75	AB1554
	Anti-Nerve Growth Factor-β	AB1526SP
	Anti-Nerve Growth Factor-β	AB1528SP
	Anti-Nerve Growth Factor-β	AB1528
	Anti-Nerve Growth Factor-β	AB1526
	Anti-Nerve Growth Factor-β	AB1526P
	Anti-Neurotrophin 3	AB1532P
	Anti-Neurotrophin 3	AB1532SP
	Anti-NGF Receptor, clone EP1039Y, rabbit monoclonal	04-1111
	Anti-NGF-β (CT), clone EP1320Y, rabbit monoclonal	04-1119
	Anti-p75 LNGFR, Saporin conjugated, clone 192	MAB390-25UG

Continued on next page

Merck Millipore offers effective solutions for research on Neurotrophic Factors:

Research Solutions	Description	Catalog No.
Neurotrophic Factor and Receptor Detection	Anti-p75 LNGFR, Saporin conjugated, clone 192	MAB390-100UG
	Anti-p75 Neurotrophin Receptor, FITC conjugated	AB15566F
	Anti-p75NTR (Neurotrophin Receptor)	07-476
	Anti-p75NTR (Neurotrophin Receptor), clone ME20.4	05-446
	Anti-phospho-TrkB (Tyr816)	ABN1381
	Anti-pro-NGF (NT), clone EP1318Y, rabbit monoclonal	04-1142
	Anti-Trk B, a.a. 54-67 rTrkB	AB5372
	Anti-TrkA	06-574
	Anti-TrkB	07-225
	Anti-TrkC	07-226
	Anti-WNT3	ABS464
Chemotaxis and Migration Assays	Pseudopodia Purification Kit	ECM660
	QCM™ Chemotaxis Cell Migration Assay, 24-well (3 µm), fluorimetric	ECM505
	QCM™ Chemotaxis Cell Migration Assay, 24-well (3µm), colorimetric	ECM504
	QCM™ Chemotaxis Cell Migration Assay, 24-well (5 µm), colorimetric	ECM506
	QCM™ Chemotaxis Cell Migration Assay, 24-well (5 µm), fluorimetric	ECM507
	QCM™ Chemotaxis Cell Migration Assay, 24-well (8 µm), colorimetric	ECM508
	QCM™ Chemotaxis Cell Migration Assay, 24-well (8 µm), fluorimetric	ECM509
	QCM™ Chemotaxis Cell Migration Assay, 96-well (3 µm), fluorimetric	ECM515
	QCM™ Chemotaxis Cell Migration Assay, 96-well (5 µm), fluorimetric	ECM512
	QCM™ Chemotaxis Cell Migration Assay, 96-well (8 µm), fluorimetric	ECM510
	QCM™ Haptotaxis Cell Migration Assay	ECM581
	QCM™ Haptotaxis Cell Migration Assay -Collagen I, 24-well, colorimetric	ECM582
	QCM™ Haptotaxis Cell Migration Assay -Fibronectin, 24-well, colorimetric	ECM580
	Quantitative Pseudopodia Assay Kit	ECM650
Neurotrophic Factor ELISA	ChemiKine™ Brain Derived Neurotrophic Factor, Sandwich ELISA	CYT306
	ChemiKine™ Nerve Growth Factor, Sandwich ELISA	CYT304
Neurotrophic Proteins	GDNF, Human Recombinant Animal Free	GF322
	Nerve Growth Factor 7.0s	NC010
	Nerve Growth Factor-β	GF028
	NT-4 Protein, Human Recombinant Animal Free	GF309
	TrkA Protein, active, 10 µg	14-571
	TrkA Protein, active, 250 µg	14-571M
	TrkB Recombinant Human Protein, Active, 10 µg	14-507
	TrkB Recombinant Human Protein, Active, 250 µg	14-507M
	β-NGF Protein, Human Recombinant Animal Free	GF307

For a complete selection, visit: www.merckmillipore.com

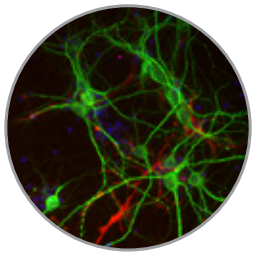
TECHNOLOGY HIGHLIGHT

The CellASIC® ONIX Microfluidic Platform

Biology is so much more than DMEM/FBS, 37°C, 5% CO_2. Living cells are constantly changing systems of interconnected mechanisms. Unlocking our understanding of these dynamic mechanisms requires real-time, instantaneous experimental control. The CellASIC® ONIX Microfluidic Platform was specifically designed to provide the dynamic cellular microenvironment control that has been missing until now.

With the flexible, intuitive CellASIC® ONIX Microfluidic Platform, you can easily take control of your cell culture. Simply program automated changes to culture media, gas and temperature, while tracking cell responses. By taking control of this truly *in vivo*-like environment, you'll be able to perform dynamic, time-lapse experiments never before possible.

Advanced control for live cell analysis. The system complements your microscope to provide a total solution for capturing the highest quality data with minimal effort.



Immunocytochemistry of primary neurons cultured, stained and imaged using the CellASIC® ONIX system. Primary rat cortical neurons were cultured to Day 15 and immunostained for MAP2 (Green, neurons) and GFAP (Red, astrocytes).

"...We've been able to quickly and easily perform novel and technologically demanding experiments without any prior microfluidic experience. I've been able to focus on the fundamental biological questions while letting CellASIC® provide me with the tools I need to answer them."

Maheshri Lab, MIT

Dynamic environmental control over live cells

Measure cellular responses to pre-programmed perfusion, temperature, and gas environment changes. The CellASIC® ONIX Microfluidic Platform automates all the necessary requirements for live cell analysis, while giving you the control to discover new science.

Microfluidic cell culture plate advantages

- Perform four independent experiments at once
- Compatible with any standard inverted microscope
- High resolution viewing though thin glass bottom
- Dynamic control over flow, gas and temperature
- Laminar flow for rapid solutions switching and stable gradient formation
- Perfusion barriers allow continuous mass transport without shear stress

You're just minutes away from acquiring data using "load-and-go" CellASIC® ONIX Microfluidic Plates. Intuitive and easy-to-program CellASIC® ONIX FG Software automates your entire customizable protocol, so you can spend more time exploring the countless experimental possibilities enabled by this single platform.

What you've always imagined for your dynamic cell biology experiments can now be a reality... using the CellASIC® ONIX Platform.

"What the CellASIC® system lets us do is very rapidly turn on and off conditions or insults while following single cells."

Dr. Ethan Garner, Harvard University

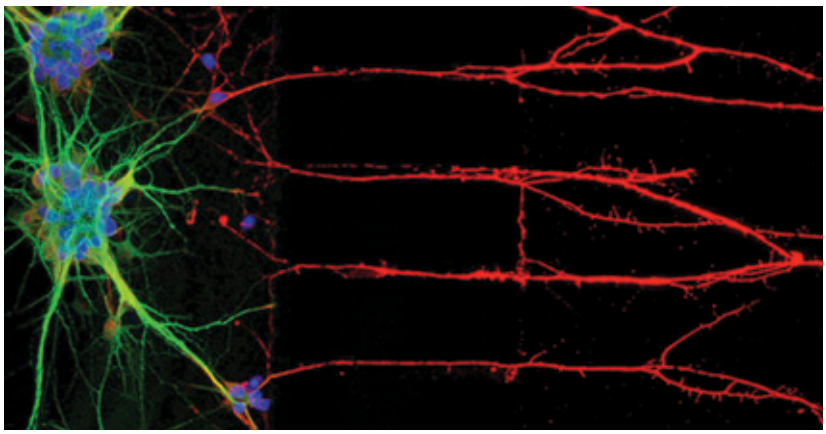
For details, visit: **www.merckmillipore.com/cellasic**

Neurite Outgrowth

Whether in early development, adult plasticity, or post-axotomy regeneration, neurons must generate neurites and make precise, distant functional connections.

Understanding the development of this network can improve therapeutics for nervous system developmental disorders and neurodegenerative disease. Although it is understood that neurons generally make connections by timely expression of growth cone receptors and axon guidance cues, the roles of genetic, epigenetic and microRNA modulation, glial signaling, scarring, and other non-neural factors are now being revealed. Regulation of extracellular matrix (ECM) degradation similarities between neural and cancer cells is also of increasing interest. New translational research is also exploring how to stabilize dystrophic growth cones in an injured spinal cord, allowing for regeneration and improved sensorimotor function.

Merck Millipore's cell culture systems and antibody design expertise has yielded products for studying neurite outgrowth, retraction, and synapse formation.



Neurite outgrowth in mouse Balb/c P3 mixed cortical neuron culture. Somas and dendrites are stained with anti-MAP2 (Catalog No. MAB3418, green). Axons are stained with anti-βIII tubulin (Catalog No. AB15708, red). Nuclei are counterstained with DAPI (blue).

Did You Know?

The length of a giraffe primary afferent axon (from toe to neck) is about 15 feet!

New directions in neurite outgrowth research

Axonal regeneration: Ruschel J, hellal F, Flynn KC, Duparz S, et al. Sytemic administration of epothilone B promotes axon regeneration after spinal cord injury. Science. 2015; 348:347-352.

Retinal projections: Erskine L, Herrera E. connecting the retina to the brain. ASN Neuro. 2014; 6. 1759091414562107

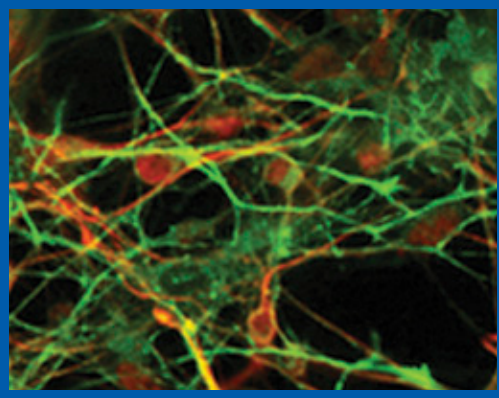
Neural/cancer invadosomes: Santiago-Medina M, Gregus KA, Nichol RH, O'Toole SM, Gomez TM. Regulation of ECM degradation and axon guidance by growth cone invadosomes. Development. 2015; 142:486-496.

ANTIBODY SPOTLIGHT

Anti-Doublecortin Antibody

Doublecortin (DCX) is a microtubule-associated protein expressed almost exclusively in immature neurons. Neuronal precursors express DCX for 2-3 weeks as the cells mature into neurons. Due to the nearly exclusive expression of DCX in developing neurons, this protein has been used increasingly as a marker for neurogenesis.

This antibody is validated for use in WB, IH, ICC for the detection of Doublecortin.



Primary neurons and astroglia from mouse forebrain stained with Anti-Doublecortin (Catalog No. AB2253, green) and Milli-Mark® pan-neuronal marker (Catalog No. MAB2300, red).

Advances in Technology

Biomaterial-based implantable scaffolds are being tested in combination with iPS cells and microenvironment changes to promote regeneration in spinal cord damage models. There are a number of promising pre-clinical studies that combine scaffolds, cells, drugs and/or nucleic acids to overcome the natural outgrowth-suppressive environment of spinal cord injury.

Pires LR, Pego AP. Regenerative Med. 2015; Epub 1-12.

Technical Tip

Use microfluidic or Boyden chamber structures to spatially restrict somas from neurites. For microfluidic separation, use at least 150 µm long channels to separate dendrites from axonal projections. For Boyden chambers, 3 µm membrane pores will prevent most types of neuron somas from migrating through. For PC12 cells, use 1 µm pore sizes.

Featured Technique:

Neurite Outgrowth Assay

The characterization of neurite formation, maturation, and collapse/resorption is an area of intense interest, since these cellular processes are essential for interconnection of neuronal cell bodies. Neurites are particularly interesting in relation to neuropathological disorders, neuronal injury/regeneration, and neuropharmacologic research and screening. The study of neurites is hampered by difficulties associated with isolating and purifying these minute structures.

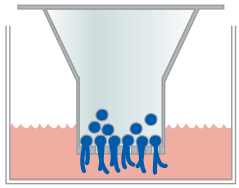
Currently available methods for measuring neurite outgrowth are dependent on manual microscopic examination of individual cells or measurement of total fluorescence from a labeled neuronal cell population using a fluorescence plate reader. The disadvantages of the first method are labor intensiveness and subjectivity. The second method does not allow for discrimination between fluorescently-labeled neuronal cell bodies and neurites. Consequently, the lack of a means to isolate and purify sufficient neurite material, and the lack of a uniform and highly reproducible method for neurite quantification, has impeded the understanding of the role of these cellular structures in development, injury, and disease states. Merck Millipore offers Neurite Outgrowth Assay Kits that allow easy measurement of neurite outgrowth.

Featured Solution:

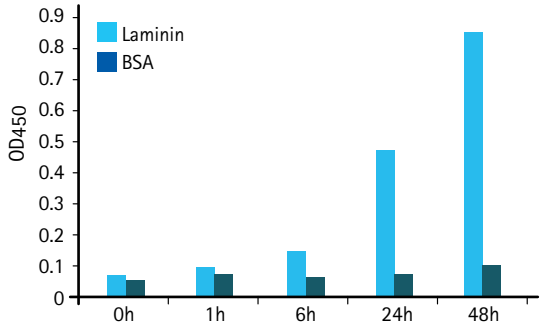
Neurite Outgrowth Assay Kit

(Catalog No. NS230)

Merck Millipore's Neurite Outgrowth Assay Plus kit (3 µm pore size) uses Millicell® microporous tissue culture inserts, which contain permeable membranes that allow separation of neurite material from cell bodies for harvesting and use in this or other biochemical analyses. Induced neurites traverse these membrane pores to enable neurite quantitation or purification from the underside of the membrane.



Neurite Outgrowth (48 hrs at 37 °C).



Neurite extension on laminin-coated, but not BSA-coated, inserts dramatically increases over time.

Other Kits:

Description	Catalog No.
Neurite Outgrowth Assay Kit (3 µm)	NS220
Neurite Outgrowth Assay Kit (1 µm)	NS225

Solutions for your Research

Merck Millipore offers effective solutions for research on Neurite Outgrowth:

Research Solutions	Description	Catalog No.
Neuronal Marker Detection	Anti-Doublecortin	AB2253
	Anti-Neural Cell Adhesion Molecule	AB5032
	Anti-Neurofilament M (145 kDa), C-terminus	AB1987
	Anti-Tubulin, βIII isoform, C-terminus, clone TU-20 (Similar to TUJ1)	MAB1637
	Guinea pig Anti-Neurexin-1-α	ABN35
	Rabbit Anti-Neurexin-1-α	ABN98
	Rabbit Anti-Pan-Neurexin 1	ABN161
Growth Factor ELISA	ChemiKine™ Brain Derived Neurotrophic Factor (BDNF) Sandwich ELISA Kit	CYT306
	ChemiKine™ Nerve Growth Factor (NGF) Sandwich ELISA Kit	CYT304
Neurite Outgrowth Assays	AXIS® Axon Isolation Device, Tissue Culture Ready, 150 µm	AX15010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 450 µm	AX45005TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 450 µm	AX45010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 900 µm	AX90010TC
	AXIS® Axon Isolation Device, Tissue Culture Ready, 6-well	AX50010TC

For a complete selection, visit: www.merckmillipore.com

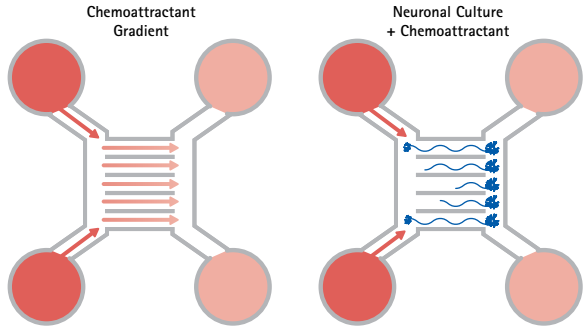
TECHNOLOGY HIGHLIGHT

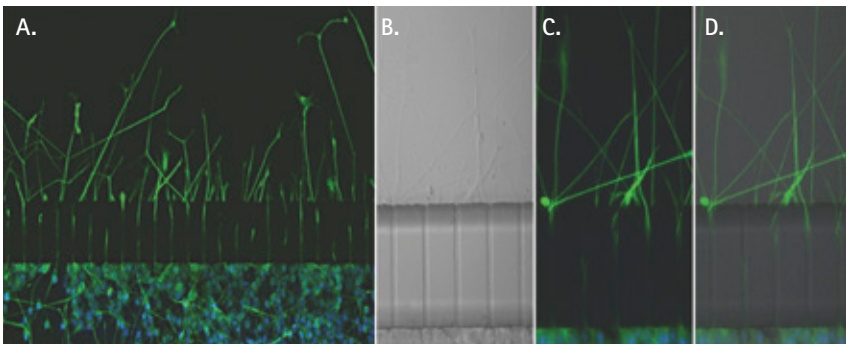
AXIS® Axon Investigation System

The AXIS® platform is Merck Millipore's most advanced tool for the study of neurite outgrowth. This slide-mounted microfluidic two-chamber system enables the deposition and culture of neural cells and the spatially controlled addition of growth factors, toxins, and other reagents. Neurite outgrowth is restricted to narrow, parallel channels, and the resultant outgrowth or collapse behavior is easily observed under a microscope. The result is a powerful platform for the study of somas, neurite outgrowth, and synapse formation.

How the AXIS® Isolation Device Works

AXIS® Axon Isolation Device is a two chamber system, each composed of two wells and an interconnected channel, separated by a set of microgrooves. The hydrostatic pressure formed by volume differential between chambers induces fluidic isolation of the solution on the low volume side of the device. The microfluidic design of an AXIS® device allows for development and maintenance of a fluidic gradient of chemoattractants, toxins or other molecules of interest, facilitating controlled exposure and differentiation of axons.





N1E-115 cells were loaded in the lower channel and cultured for 5 days in differentiation media. The cells were then fixed and stained with DAPI (blue) and with the neuronal cell stain MAB2300X (green).

A) fluorescent image showing the N1E-115 cells differentiating through the microgrooves of the AXIS® device. Note that the cell bodies (somas) are entirely contained on one side of the device and only the neurites are extended through the microgrooves into the other channel.
B) higher resolution white light image of the cells and device.
C) corresponding fluorescent image.
D) overlay of images B and C to verify that the neurites extend through the microgrooves only.

	AXIS® Axon Isolation Device Microgroove Length	Tissue Culture Ready (TC)	Plasma Bonded (PB)	Plasma Bonded to Confocal dishes (PBC)
Optimal Use		Ready-to-use devices. Just mount on slide and go. Good for slide or coverslip users.	Pre-sealed to glass slides to prevent leakage. Excellent for beginners.	Pre-sealed to coverslip glass culture dishes for confocal imaging from bottom.
Short experiments or maximal growth in axonal compartment	150 µm, 4 well	AX15010TC	AX15005PB	AX15005PBC
Most common axonal studies	450 µm, 4 well	AX45010TC	AX45005PB	AX45005PBC
More complex studies where 2 sets of microgrooves are needed	500 µm, 6-well	AX50010TC	AX50005PB	AX50005PBC
Longer axonal growth or transport studies	900 µm, 4 well	AX90010TC		

For details, visit: www.merckmillipore.com/axis

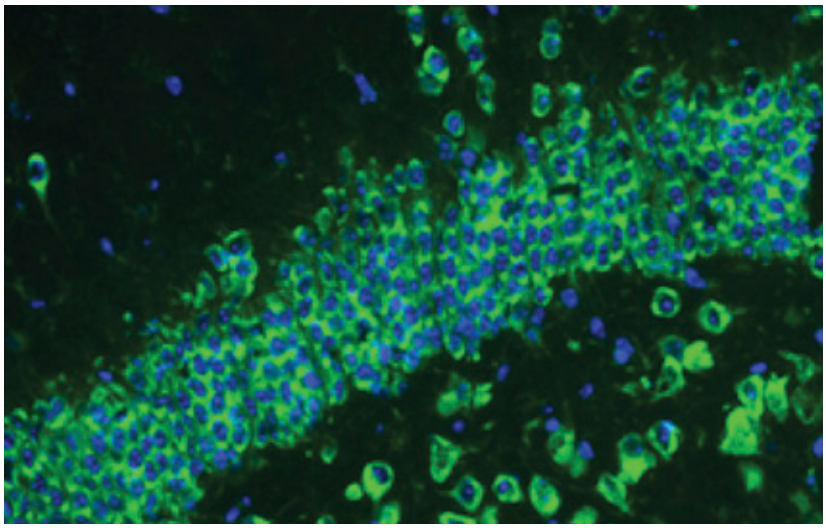
Transmitters, Receptors, Ion Channels & Transporters

These components mediate neural excitation and modulate signal transmission at the synaptic cleft.

Transmitter release, ion channel dynamics, receptor function, and neurotransmitter transport are extensively studied to gain insight into learning, memory, addictions, and many neurological diseases. Not surprisingly, the vast majority of all neuropharmacological agents available today act on these synaptic targets.

Researchers are now looking at neurotransmission and synaptic receptor function from new angles. What are the epigenetic influences on signal transduction? What roles do receptor polymorphisms play in normal and disease states? How do neural-glial interactions impact transmission? New precision control techniques, such as CRISPR and optogenetics coupled with classical applications like live cell imaging and immunodetection are giving researchers greater insights into molecular and cellular control of neural processes and neurotransmission.

Merck Millipore continues to develop precision pan- and phosphospecific antibodies and assays to elucidate the relationship between neurotransmitter transport, membrane depolarization, and neuroexcitability.



Staining of adult mouse brain (8 µm coronal cryosections) with Anti-GluR2, AlexaFluor® 488 Conjugate (Catalog No. MAB397A4, green). Nuclei are stained with Hoechst 33342 (blue).

Did You Know?

The mongoose of Herpestidae family has higher resistance to cobra α-bungarotoxin venom because the mongoose (and cobra) muscle-type nicotinic acetylcholine receptors differ from other prey mammals in just four key amino acid positions.

New directions in neural transmission research

Magnetothermal neuromodulation: Chen R, Romero G, Christiansen MG, Mohr A, Anikeeva P. Wireless magnetothermal deep brain stimulation. Science. 2015; 27:1477-1480.

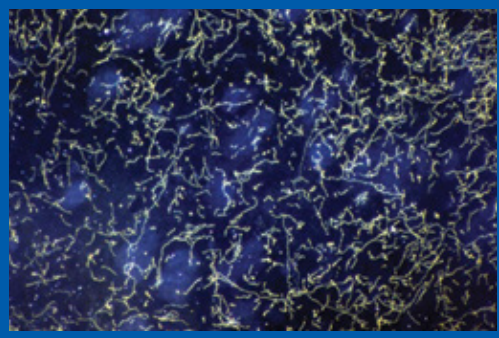
Neuroglial transmission: Gundersen V, Storm-Mathisen J, Bergersen LH. Neuroglial Transmission. Physiol Rev. 2015; 95: 695-726.

Polymorphisms and disease: Moreau C, Meguig S, Corvol J-C et al. Polymorphism of the dopamine transporter type 1 gene modifies the treatment response in Parkinson's disease. Brain. 2015; 138:1271-1283.

ANTIBODY SPOTLIGHT

Transporter Antibodies

Merck Millipore's numerous antibodies against re-uptake-related proteins are often used as postsynaptic or perisynaptic markers. Merck Millipore offers a broad portfolio of over 150 specific antibodies to transporters including those for glutamate, dopamine, serotonin, norepinephrine, glycine, choline, calcium, glucose, and more.



Staining of 5-HT fibers in normal rat striatum using Anti-Serotonin Transporter Antibody (Catalog No. AB9726).

Advances in Technology: Optogenetics

Optogenetics is a technique that allows researchers to modulate the activity of target neurons using specific wavelengths of light. This is accomplished by coupling genetic expression of light-sensitive channel proteins within targeted cells to precision light exposure and measurement. The key reagents used in optogenetics are light-sensitive proteins. Several types of microbial opsins, are currently used to modulate neuronal function. Each type is sensitive to a different range of wavelengths and responds by altering different ion conductances. Blue light is commonly used to stimulate channelrhodopsin-2 (ChR2), a nonselective cation channel, resulting in potassium, sodium, and calcium flow, which depolarizes the neuron to threshold. Other channelrhodopsin variants are now available that differ in depolarization and wavelength sensitivity characteristics yielding even finer control over neural modulation. The halorhodopsin (NpHR) class of chloride pumps, which respond to yellow light, are typically used to hyperpolarize neural membranes and induce inhibitory responses. The class of photoactivatable proton pumps, exemplified by archaerhdopsin-3 (Arch) also create hyperpolarization signals.

Guru A, Post RJ, Ho Y-Y, Warden MR. Int J Neuropsychopharmacol. 2015; July 25; Epub 1-8.

Technical Tip

The acceptance of optogenetics in the scientific community as an effective tool for modulating neural function or cellular signaling has come from careful verification of effects with traditional, well established tools. Electrodes are routinely used to record neural function during stimulation. Sequencing verifies the construct insertion. Protein presence and downstream protein-protein interactions and cell signaling are measured using antibodies and antibody-based technologies such as Western blotting and immunohistochemistry.

Featured Technique:

Inhibition and Activation

The inhibition or activation of specific proteins can facilitate the understanding of their biological function. Small molecules, including inhibitors and activators are critical tools for researchers studying cell signaling, neurotransmission, and other mechanisms that regulate cellular processes.

Merck Millipore's Calbiochem® brand of high quality small molecules, including inhibitors, agonists, antagonists, activators, channel blockers and openers, have been cited in thousands of peer-reviewed publications. Choose from a wide selection of high purity small molecules to excite your research.

Featured Solution:

Calbiochem® brand of high quality small molecules

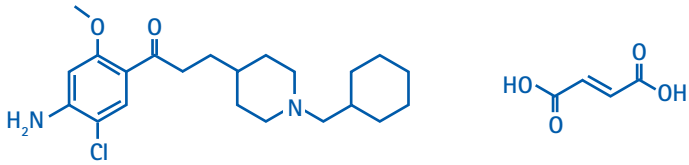
Donecopride Fumarate

(Catalog No. 532383)

Also known as: Serotonin receptor (5-HT4R) agonist, AChE Inhibitor, MR31147

A cell permeable, brain penetrating piperidin-4-yl-propanone compound that acts as a highly potent, selective and partial agonist of serotonin subtype 4 receptor ((h)5-HT4R; K_i = 10.4 nM and 48% efficacy compared to 5-HT control). Also acts as a potent, mixed type, competitive inhibitor of acetylcholinesterase (IC_{50} = 16 nM for human AChE) and offers selectivity over butyrylcholinesterase (IC_{50} = 3.5 µM). With respect to 5-HT2BR, it behaves like an inverse agonist (K_i = 1.6 nM). Shown to have a precognitive effect in murine model where it significantly improves discrimination index.

Reference overview: Lecoutey, C., et al. 2014. Design of donecopride, a dual serotonin subtype 4 receptor agonist/acetylcholinesterase inhibitor with potential interest for Alzheimer's disease treatment. Proc. Natl. Acad. Sci. USA 111, E3825–E3830.



Solutions for your Research

Merck Millipore offers effective solutions for research on Neurotransmission:

Research Solutions	Description	Catalog No.
Transmitter Detection	Anti-Dopamine β Hydroxylase-SAP, clone 4F10.2	MAB394-25UG
	Anti-GABA	ABN131
	Anti-Glutamate	AB133
	Anti-Glutamate	MAB5304
	Anti-Glutamate (No Glutaraldehyde)	AB5018
	Anti-Serotonin	AB125
	Anti-Serotonin	AB938
Receptor Markers	Anti-Acetylcholine Receptor β, clone 124	MABS452
	Anti-Acetylcholine Receptor-γ	AB5936
	Anti-Acetylcholine Receptor-ε	AB5938
	Anti-Capsaicin Receptor	MAB5568
	Anti-Capsaicin Receptor (Ab-1) (824-838) Rabbit pAb	PC420
	Anti-Capsaicin Receptor, CT	AB5566
	Anti-Capsaicin Receptor, CT	AB5370
	Anti-Capsaicin Receptor, NT	AB5889
	Anti-CHRNA5	ABN503
	Anti-Dopamine D(1A) Receptor	ABN20
	Anti-Dopamine D2 Receptor	ABN462

Continued on next page

Merck Millipore offers effective solutions for research on Neurotransmission:

Research Solutions	Description	Catalog No.
Receptor Markers (continued)	Anti-Dopamine D2 receptor (DRD2), clone 3D9	MABN53
	Anti-Dopamine D3 receptor, clone N331/19	MABN463
	Anti-Dopamine D_5 Receptor Rabbit pAb	324408
	Anti-GABA(A)R BETA 1 Protein, clone N96/55	MABN498
	Anti-GABA(B)R1 Protein, clone N93A/49	MABN492
	Anti-GluR1	ABN241
	Anti-GluR1, clone C3T, Rabbit monoclonal	04-855
	Anti-GluR1, N-terminus, clone RH95	MAB2263
	Anti-GluR1, Recombinant, Rabbit monoclonal	05-855R
	Anti-GluR2	AB1768
	Anti-GluR2, clone 6C4, Alexa Fluor® 488 Conjugate	MAB397A4
	Anti-GluR2, clone L21/32	MABN71
	Anti-GluR2, extracellular, clone 6C4	MAB397
	Anti-GluR3, clone 3B3	MAB5416
	Anti-GluR4	AB1508
	Anti-GluR5	07-258
	Anti-Glutamate Receptor 5, 6, 7, clone 4F5	MAB379
	Anti-Glutamate Receptor Delta 1/2	AB2285
	Anti-KA2/GRIK5 (Kainate Receptor)	06-315
	Anti-mGluR1	07-617
	Anti-mGluR2	07-261-I
	Anti-mGluR2/3	06-676
	Anti-mGluR4	AB15097
	Anti-mGluR5, clone 11D9.1	MABN139
	Anti-mGluR5, clone N75/33	MABN540
	Anti-mGluR7	07-239
	Anti-mGluR8	AB10556
	Anti-Muscarinic Acetylcholine Receptor m1	AB5164-200UL
	Anti-Muscarinic Acetylcholine Receptor m2 Antibody	AB5166-200UL
	Anti-Muscarinic Acetylcholine Receptor m4, clone 18C7.2	MAB1578
	Anti-Neuronal acetylcholine receptor α-7, clone 6F12.2	MABN529
	Anti-Nicotinic Acetylcholine Receptor α3	AB15320
	Anti-Nicotinic Acetylcholine Receptor α4	AB15322
	Anti-Nicotinic Acetylcholine Receptor α4	AB5590
	Anti-Nicotinic Acetylcholine Receptor α7	AB15332
	Anti-Nicotinic Acetylcholine Receptor β2	AB15325
	Anti-NMDAR 2A, clone 2F6	MAB5530
	Anti-NMDAR1 Splice Variant C1	AB5046P
	Anti-NMDAR1 Splice Variant C2	AB5048P
	Anti-NMDAR1, (all splice variants), clone R1JHL	MAB1586
	Anti-NMDAR1, C Terminus, Alexa Fluor®488 Conjugate	05-432A4
	Anti-NMDAR1, clone 54.1	MAB363

Continued on next page

Merck Millipore offers effective solutions for research on Neurotransmission:

Research Solutions	Description	Catalog No.
Receptor Markers (continued)	Anti-NMDAR2A	MAB5216
	Anti-NMDAR2B	MAB5780
	Anti-NMDAR2B phospho Tyr1252	AB9630
	Anti-NMDAR2B phospho Tyr1336	AB9690
	Anti-NMDAR2B, clone 13A11	MAB5220
	Anti-NMDAR2C	MAB5576
	Anti-NMDAR2D	MAB5578
	Anti-phospho GluR1 (Ser831)	AB5847
	Anti-phospho GluR1 (Ser845)	AB5849
	Anti-phospho GluR1 (Thr840)	AB2292
	Anti-phospho-GluR2 (Ser880)	07-294
	Anti-Serotonin 2A (5-HT2A) Receptor (22-41) Rabbit pAb	PC176
	Anti-Serotonin 3 (5-HT3) Receptor (444-457) Rabbit pAb	PC347
	Anti-Serotonin Receptor 1A	AB15350
	Anti-Serotonin receptor 1A, clone 19A9.2	MAB11041
	Anti-Serotonin Receptor 1E	AB9101
	Anti-Serotonin Receptor 1F	AB9402
	Anti-Serotonin Receptor 7	AB9405
GPCR Dynamics	ChemiScreen™ GPCR Membrane Preparations	Various
	ChemiBrite™ GPCR Frozen Cells	Various
Ion Channel Localization	Anti-Cav3.1 Ca^{2+} channel, clone N178A/9	MABN464
	Anti-Cav3.2 Calcium Channel Subunit, clone N55/10	MABN487
	Anti-HCN1	AB5884-200UL
	Anti-HCN2	AB5378-50UL
	Anti-KCNQ1 Potassium Channel, clone N31A/10	MABN55
	Anti-Kvβ1, clone K40/17	MABN658
	Anti-Potassium Channel KCNQ5	ABN1372
	Anti-Potassium Channel Kv1.2, clone K14/16	MABN77
	Anti-Potassium Channel Kv1.3, clone L23/27	MABN76
	Anti-Potassium Channel Kv10.1, clone 56	MABN378
	Anti-Potassium Channel KvLQT1, CT	AB5932
	Anti-Potassium Channel Kvβ2	MABN652
	Anti-SLC6A6 Antibody	ABN750
	Anti-Sodium Channel Nav1.8, pain	AB9274
	Anti-Sodium Channel, Voltage Gated, Brain Type II	AB5206
Ion Channel Function	PrecisION™ Recombinant Stable Cell Lines	Various
	PrecisION™ Ion Channel Membrane Preparations	Various
	Ready-to-Assay™ Ion Channel Frozen Cells	Various

Continued on next page

Merck Millipore offers effective solutions for research on Neurotransmission:

Research Solutions	Description	Catalog No.
Transporter Detection	Anti-Calcium Transporter 1	AB15500
	Anti-Choline Transporter	ABN458
	Anti-Dopamine Transporter	AB15344
	Anti-Dopamine Transporter	AB2231
	Anti-Dopamine Transporter, clone mAb16	MABN669
	Anti-Dopamine Transporter, clone mAb16	MABN669
	Anti-Excitatory Amino Acid Transporter (GLT-1) Rabbit pAb	PC154
	Anti-Glutamate Transporter, Glial	AB1783
	Anti-Glutamate Transporter, neuronal	AB1520
	Anti-Glycine Transporter 2, neuronal	AB1773
	Anti-Glycine Transporter 2, neuronal	AB1771
	Anti-$Na^+K^+Cl^-$ Cotransporter 2	AB2281
	Anti-Norepinephrine Transporter	AB2234
	Anti-Serotonin Transporter	AB10514P
	Anti-SLC30A8	ABS767
	Anti-SLC38A3, clone 1A10.2	MABN316
	Anti-SLCO1B3	ABS675
	Anti-Vesicular Acetylcholine Transporter (VAChT)	ABN100
Related Small Molecules	(+)-MK 801 Maleate	475878
	(2S,4R)-4-methylglutamate	505019
	(S)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid	155305
	ATPA	505018
	Cinnabarinic acid	504820
	CNQX	504914
	CX546	505016
	D-(–)-2-Amino-5-phosphonopentanoic Acid	165304
	EGLU ((2S)-α-Ethylglutamic acid)	504378
	Homoquinolinic acid	504483
	Kainic Acid	420318
	Kir1.1 Inhibitor, VU591	422682
	Kir2.1 Inhibitor, ML133	422689
	Kynurenic Acid Sodium Salt	505027
	L-(+)-2-Amino-4-phosphonobutyric Acid	165300
	L-Serine-O-phosphate	509705
	mGlu7 Antagonist, XAP044	531145
	mGluR5 Antagonist, MTEP	445874
	mGluR5 Ligand, CDPPB	445865
	N-Methyl-D-aspartic Acid, Hydrate	454575
	NMDA Antagonist XI, N1-dansyl-spermine	505764
	NMDAR2C/2D Inhibitor, DQP-1105	454586
	PICK1 PDZ Domain Inhibitor	529531
	TrpA1 Antagonist, HC-030031	648485
Other Tools	Blood-Brain Barrier hCMEC/D3 Cell Line	SCC066

For a complete selection, visit: www.merckmillipore.com

TECHNOLOGY HIGHLIGHT

Millicell® Plates and Slides

Membrane-Based Cell Assays

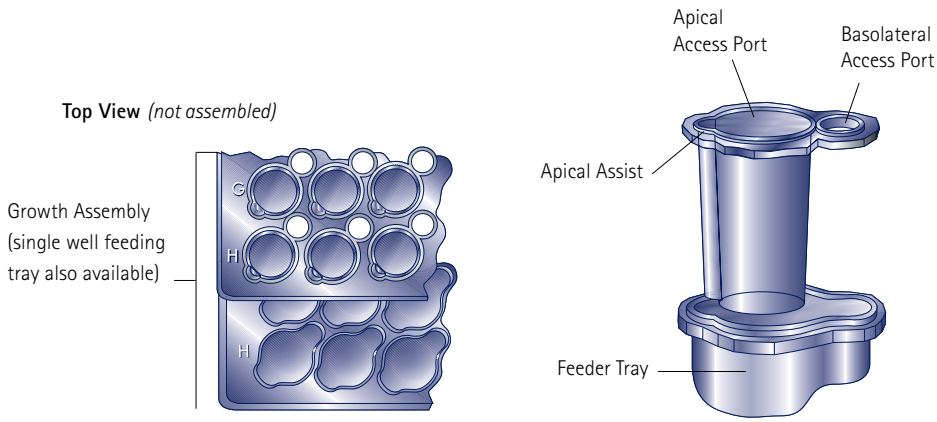
Smart design for smart research.

For studying the effects of small molecules, as well as the roles of growth factors such as cytokines, signal transduction factors such as kinases and phosphatases, and other bioactive compounds, use Millicell® membrane-based cultureware to promote the natural growth of adherent and suspension cell lines. The optimized membranes result in cells with structure and function that more closely mimic their *in vivo* counterparts, leading to more biologically relevant results for applications including primary and secondary screening, transport assays, toxicity screening, cell signaling, cell proliferation, and ADME drug safety studies.

The Millicell® Cell Culture product family includes 24-well and 96-well insert plates, as well as hanging and standing single-well inserts. Each platform is available with a selection of membranes to support a range of applications.

- Optimized membranes for reliable monolayer formation
- Transparent membranes for easy cell growth monitoring
- Choice of device platforms available

Millicell® multiwell cell culture plates

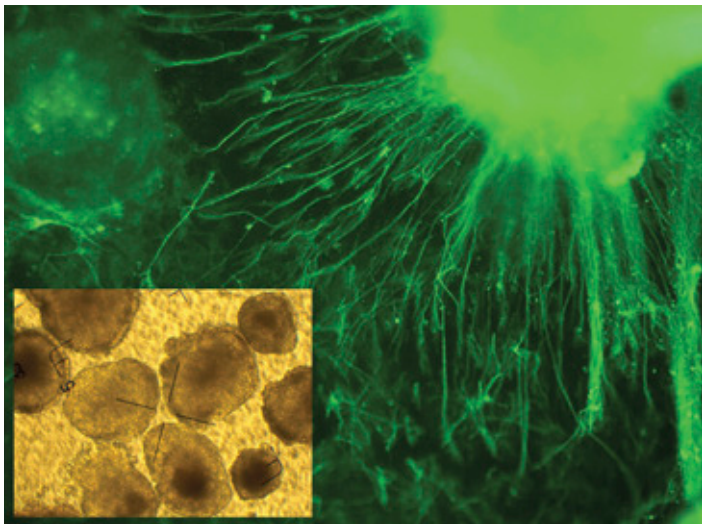


These automation-compatible plates incorporate a patented design to maintain assay integrity and prevent monolayer disruption, contamination or damage during analysis. The 96-well growth assemblies include a choice of a 96-well or single-well feeder trays. The format is also available in a 24-well design.

For more information, visit: www.merckmillipore.com/millicell

Plates and Inserts by Application Type

Recommended membranes and pore sizes



Neuron differentiation of embryonic stem cells in Millicell® -24, 1 µm PET filter plates. Murine embryonic stem cells were formed into suspended embryoid bodies (EBs), then transferred to Millicell® -24, 1 µm PET for attachment and differentiation. Neural differentiation after retinoic acid treatment of attached EBs was confirmed by anti-neurofilament immunofluoresence. (Inset: Inverted phase contrast imaging through membrane of live EBs in medium.)

Filter Codes

Code	Membrane Type	Membrane Material
CM	Biopore™	Hydrophilic PTFE
HA	MF-Millipore™	Mixed cellulose esters
PCF	Isopore™	Polycarbonate
PET	PET	Polyethylene terephthalate

Application	Standing Insert (pore size)	Hanging Insert (pore size)	24-Well Plate (pore size)	96-Well Plate (pore size)
Angiogenesis	PCF (3, 8)	PET (3, 5, 8)	PCF (3, 5, 8)	MultiScreen® MIC Plate (3, 5, 8)
Cell Proliferation	PCF (0.4)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Cell Surface Receptors	PCF (0.4) HA (0.45) CM (0.4)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Chemotaxis	PCF (3, 8)	PET (3, 5, 8)	PCF (3, 5, 8)	MultiScreen® MIC Plate (3, 5, 8)
Coculture	PCF (0.4) CM (1)	PET (0.4, 1)	PET (1) PCF (0.4)	PCF (0.4) PET (1)
Migration/Invasion	PCF (8,12)	PET (5, 8)	PCF (5, 8)	MultiScreen® MIC Plate (5, 8)
Epithelial Cell Growth	PCF (0.4) HA (0.45)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Feeder Layers	PCF (0.4, 3, 8)	PET (all)	PCF (all) PET (1)	PCF (0.4) PET (1)
Fluorescent Detection/ Immunohistochemistry	PCF (all) CM (0.4)	PET (all)	PCF (all) PET (1)	PCF (0.4) PET (1)
In Vitro Fertilization	CM (0.4)	PET (1)	PET (1)	PET (1)
In Vitro Toxicology	PCF (0.4) CM (0.4) HA (0.45)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Microbial Attachment	PCF (0.4) CM (0.4) HA (0.45)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Organotypic	Organotypic (0.4)			
Phase Contrast Microscopy	CM (0.4)	PET (1)	PET (1)	PET (1)
Polarized Protein Secretions	PCF (0.4) CM (1)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Polarized Uptake	PCF (0.4) CM (0.4) HA (0.45)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Transport/Permeability	PCF (0.4)	PET (0.4, 1)	PCF (0.4) PET (1)	PCF (0.4) PET (1)
Tumor Cell Metastasis and Invasion	PCF (8,12)	PET (5, 8)	PCF (5, 8)	MultiScreen® MIC Plate (5, 8)

Product Selection

Membrane	Pore Size	Device Size	Qty/Pk	Catalogue No.
Millicell® Single-Well Standing Inserts				
Organotype insert** Biopore™ (PTFE)	0.4 µm	6-well	50	PICMORG50
HA insert MF-Millipore™ (mixed cellulose esters)	0.45 µm	6-well 24-well	50 50	PIHA03050 PIHA01250
CM insert** Biopore™ (PTFE)	0.4 µm	6-well 24-well	50 50	PICM03050 PICM01250
PCF insert Isopore (polycarbonate)	0.4 µm 1 µm 3 µm 8 µm 12 µm	6-well 24-well 24-well 24-well 24-well	50 50 50 50 50	PIHP03050 PIHP01250 PITP01250 PI8P01250 PIXP01250
Millicell® Single-Well Hanging Inserts				
PET	0.4 µm 1.0 µm 3.0 µm 5.0 µm 8.0 µm	6-well	48	MCHT06H48 MCRP06H48 MCSP06H48 MCMP06H48 MCEP06H48
PET	0.4 µm 1.0 µm 3.0 µm 5.0 µm 8.0 µm	12-well	48	MCHT12H48 MCRP12H48 MCSP12H48 MCMP12H48 MCEP12H48
PET	0.4 µm 1.0 µm 3.0 µm 5.0 µm 8.0 µm	24-well	48	MCHT12H48 MCRP24H48 MCSP24H48 MCMP24H48 MCE24H48
Millicell®-24 Cell Culture Plate Assemblies				
24-well cell culture plate, single-well feeder tray, 24-well receiver tray, and lid	PCF PET PCF PCF PCF	0.4 µm 1 µm 3 µm 5 µm 8 µm	1	PSHT010R1 Stem Cell TESTED PSRP010R1 PSST010R1 PSMT010R1 PSET010R1
24-well cell culture plate, 24-well receiver tray, and lid	PCF PCF PCF	3 µm 5 µm 8 µm	5	PSST010R5 PSMT010R5 PSET010R5
24-well cell culture plate, single-well feeder tray, and lid	PCF PET	0.4 µm 1 µm	5	PSHT010R5 Stem Cell TESTED PSRP010R5
Millicell®-96 Cell Culture Plate Assemblies				
96-well cell culture plate, single-well feeder tray, 96-well receiver tray, and lid	PCF PET	0.4 µm 1 µm	1	PSHT004R1 PSRP004R1
96-well cell culture plate, 96-well receiver tray, and lid	PCF	0.4 µm	5	PSHT004S5
96-well cell culture plate, single-well feeder tray, and lid	PCF PET	0.4 µm 1 µm	5	PSHT004R5 PSRP004R5

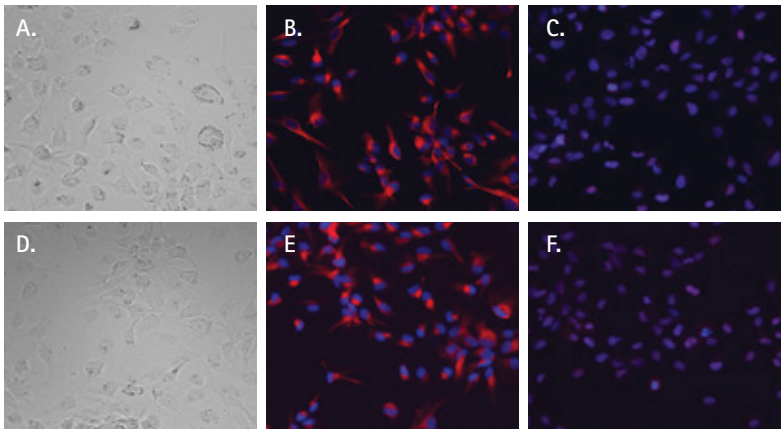
** For adherent cells, this membrane needs to be coated with an extracellular matrix.

Membrane	Qty/Pk	Catalogue No.
Tissue Culture Treated Plates		
6-well cell culture plate, tissue culture treated, sterile	50	PIMWS0650
12-well cell culture plate, tissue culture treated, sterile	50	PIMWS1250
24-well cell culture plate tissue culture treated, sterile	50	PIMWS2450
Millicell® Electrical Resistance System		
Millicell® ERS-2 Voltohmmeter		MERS00002
Replacement Electrodes		MERSSTX01
Replacement Test Electrodes		MERSSTX04
Adjustable Electrodes		MERSSTX03
Specialized Electrodes (for Millicell®-96 well plate only)		MERSSTX00
Replacement Battery 6V NiMH 2200mAH		MERSBAT01
Media Filtration Products		
Stericup®-GP filter unit, PES membrane	12	SCGPU01RE Stem Cell TESTED
Sterile Millex®-GP filter unit, PES membrane	50	SLGP033RS
Steriflip®-GP filter unit, PES membrane	25	SCGP00525

TECHNOLOGY HIGHLIGHT

Millicell® EZ SLIDE

Simplify your cell analysis by using the Millicell® EZ SLIDE to culture, fix, stain and view your sample all in one device. There's no need to remove the medium chamber from the slide prior to fixing or staining. Unique, breakable tabs means you can easily remove wells without worrying about breaking slides or harming cells or messy glue or gasket residues. Acquire data simply and quickly with Millicell® EZ SLIDES.



ReNcell® CX cells cultured on Millicell® 8-well glass EZ slides (A, B, C) and Brand B 8-well glass chambered slides (D, E, F). Cells show staining for nestin (red, B and E) and Sox-2 (red, C and F). Nuclei (blue) are stained with DAPI.

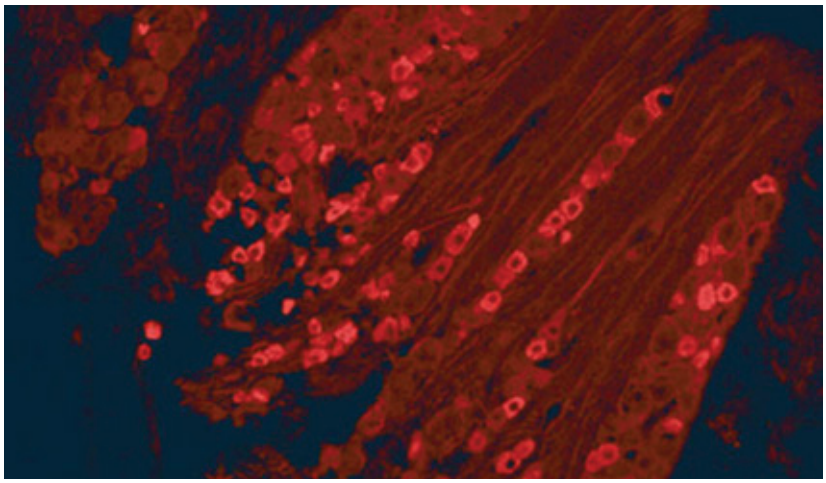
Description	Catalog No.
Millicell® EZ SLIDE (4-well glass, 16 pack)	PEZGS0416
Millicell® EZ SLIDE (4-well glass, 96 pack)	PEZGS0496
Millicell® EZ SLIDE (8-well glass, 16 pack)	PEZGS0816
Millicell® EZ SLIDE (8-well glass, 96 pack)	PEZGS0896
Millicell® EZ SLIDE Microscope Slide Holder	PEZXMSH01

For details, visit: www.merckmillipore.com/ezslide

Sensory Systems & Control

Sensory system research focuses on peripheral and central processing mechanisms in the visual, auditory, olfactory, gustatory, and pain sensing systems.

Steady advances in the understanding of the molecular genetics of retinal function are leading to novel therapeutic approaches to solving visual abnormalities. Understanding genetic variations is also shedding light on molecular mechanisms of neuropathies and channelopathies. As part of autonomic sensory control, the hypothalamus regulates and maintains homeostasis, stress response, blood pressure, glucose levels, and satiety. Indeed, a recent explosion of research supporting a much stronger neural role in weight management has led many to see obesity as a brain dysfunction. In addition, newly considered factors, such as gut microbiota signaling are also proving to be altering neurochemistry. Merck Millipore's comprehensive set of antibodies, assays, and multiplexing kits target soluble factors, hormones, receptors, and associated proteins involved in sensory systems and metabolism.



Staining of rat dorsal root ganglion using Anti-Capsaicin Receptor Antibody, CT (Catalog No. AB5566, red).

Did You Know?

Birds can eat the hottest peppers in the world with no discomfort because they lack capsaicin receptors. Capsaicin is thought to be a defensive agent against mammals after first evolving as an anti-fungal agent.

New directions in sensory research

Food addiction: Ziauddeen H, Alonso-Alonso M, Hill JO, Kelley M, and Khan NA. Obesity and the Neurocognitive Basis of Food Reward and the Control of Intake. Adv. Nutr. 2015; 6:474-486.

Gut microbiome affects brain: Allen-Bevins CR, Sela DA, Hinde K. Milk bioactives may manipulate microbes to mediate parent–offspring conflict. Evol. Med. Public Health 2015; 1:106-121.

Molecular pain mechanisms: Veldhuis NA, Poole DP, Grace M, McIntyre P, Bunnett, NW. The G Protein–Coupled Receptor–Transient Receptor Potential Channel Axis: Molecular Insights for Targeting Disorders of Sensation and Inflammation. Pharmacol. Rev. 2015; 67:36-73.

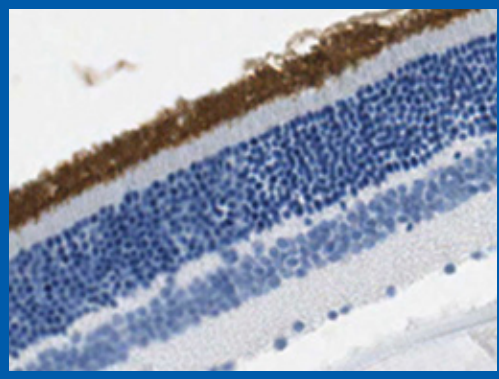
ANTIBODY SPOTLIGHT

Vision and the Retina

Cyclic nucleotide-gated channel α-1 (CNGA1) is involved in visual signal transduction, which is mediated by a G-protein coupled signaling using cGMP as second messenger. CNGA1 is activated by cyclic GMP to open cation channels which lead to a depolarization of rod photoreceptors. CNGA1 defects can cause retinitis pigmentosa type 49 (RP49).

CNGA1 is also known as cGMP-gated cation channel α-1, Cyclic nucleotide-gated cation channel 1, Cyclic nucleotide-gated channel, photoreceptor, and Rod photoreceptor cGMP-gated channel subunit α.

Envision your research with Merck Millipore antibodies for Vision, Pain, Metabolism, and more.



Mouse retina tissue was stained using Anti-CNGA1/3, clone L36/12 (Catalog No. MABN468). Positive membrane/cytoplasmic staining was observed in rods and cones.

Advances in Technology

Retinal Pigment epithelial cells derived from pluripotent hESCs are currently being used in clinical trials for age related macular degeneration. In these trials, suspensions of up to 150,000 cells are injected into an area between the degenerating photoreceptor cell and RPE cell layers. Inducible pluripotent cells are also being used. In 2014 a Japanese woman in her 70s was the first person to receive a transplanted layer of iPSC-RPE derived from her own skin cells.

Forest DL, Johnson LV, Clegg DO. Dis Model Mech. 2015; 8:421-427.

Technical Tip

Sensory structures pose challenges when preparing for immunohistochemical or *in situ* hybridization analysis in general. Preserving retinal integrity is particularly challenging. Whether to fix whole eyes, punched or not punched, or an eye cup prep is often debated. Choice of fixation and embedding medium can also play a role. The general consensus is to limit handling and thereby deforming of the eye, use a sharp thin syringe needle (23G) to puncture the cornea, and then immerse in 4% PFA in PBS.

Featured Technique:

ELISA

ELISA kits are a fast, sensitive tools for measuring relative levels of total and phosphorylated signaling proteins with phospho-specific antibodies. Researchers can quantitate the phosphorylation states of key signaling proteins, second messengers transmitting intracellular signals, and apoptosis pathway proteins using ELISA kits , often in less than five hours, and with minimal hands-on time.

Researchers rely on Merck Millipore's 25 years of experience in developing ELISAs, RIAs, and other single protein assays for sensory system, metabolic control, and cytokine biomarkers. Each assay undergoes stringent validation with high lot-to-lot consistency, inter- and intra-assay precision. Standards are validated to match reference lots—in many cases, World Health Organization standards are available and used for final quality control testing.

Precisely quantify soluble biomarkers in sera and lysates using trusted ELISA kits from Merck Millipore.

Featured Solution:

Human Neuropeptide Y (NPY) ELISA

(Catalog No. EZHNPY-25K)

Neuropeptide Y (NPY) is a 36-amino acid peptide found primarily in the brain. It has been associated with a number of physiologic processes, including the regulation of energy, balance, memory and learning. NPY is secreted by the hypothalamus, and in addition to increasing appetite, it increases the proportion of energy stored as fat and blocks signals to the brain. NPY is closely related to other peptide hormones such as pancreatic polypeptide, PYY and correlates nicely to Leptin levels.

Merck Millipore's 96-well NPY ELISA kit requires no sample extraction and supports our commitment to the study of various metabolic disease markers.

Get a complete picture of metabolism and endocrinology with sensitive, specific and reliable quantitation of circulating biomarkers. Our broad range of ELISA kits can help elucidate therapeutic mechanisms of action, establish early diagnosis of disease, predict toxicity, and more, particularly for studies of metabolic disease.

Also available:

Description	Species	Catalog No.
α-Synuclein	Human/Rat/Mouse	NS400
Amyloid β, 1-40	Human	EZHS40
Amyloid β, 1-42	Human	EZHS42
Amyloid β, SET	Human	EZHS-SET
Amyloid β (Brain), 1-40	Human	EZBRAIN40
Amyloid β (Brain), 1-42	Human	EZBRAIN42
Amyloid β (Brain), Set	Human	EZBRAIN-SET
Brain Derived Neurotrophic Factor (BDNF)	Human/Rat	CYT306
Glial Fibrillary Acidic Protein (GFAP)	Human/Rat/ Mouse	NS830
Nerve Growth Factor (NGF)	Rat/Mouse	CYT304
Neuropeptide Y (NPY)	Rat/Mouse	EZRMNPY-27K
Phosphorylated Neurofilament (pNF-H)	Multi-species	NS170
Pigment Epithelium Derived Factor (PEDF)	Human	CYT420
S100B	Human	EZHS100B-33K

For a complete selection, visit: www.merckmillipore.com/elisa

Solutions for your Research

Merck Millipore offers effective solutions for research on Sensory Systems and Control:

Research Solutions	Description	Catalog No.
Sensory and Metabolic Target Detection	Anti-α7 Nicotinic Receptor, N-Terminus	AB5637
	Anti-Arrestin, visual	MAB5580
	Anti-Brain-Derived Neurotrophic Factor (BDNF)	AB1779SP
	Anti-Capsaicin Receptor	MAB5568
	Anti-Cocaine and Amphetamine Related Transcript	AB5340P
	Anti-DARPP-32, phosphoThr75	AB9208
	Anti-Dopamine Transporter, N-terminus, clone DAT-nt	MAB369
	Anti-Ghrelin, Active	AB9756
	Anti-mPER1 (residues 6-21)	AB2201
	Anti-NMDAR2A and B	AB1548
	Anti-Opsin, Blue	AB5407
	Anti-Orexin-1 Receptor	AB3092
	Anti-P2X3 Receptor	AB5896
	Anti-PER2	AB2202
	Anti-PYY, amino acids 24-36	AB15666
	Anti-Rhodopsin, clone C7	MAB2236
	Anti-Substance P Receptor	AB5060
Sensory System and Metabolism Assays	Human Neuropeptide Y (NPY) ELISA	EZHNPY-25K
	Human PYY (3-36) Specific RIA	PYY-67HK
	Human PYY (Total) ELISA	EZHPYYT66K
	Human PYY (Total) RIA	PYYT-66HK
	MILLIPLEX® MAP Canine Pituitary Magnetic Bead Panel	CPTMAG-96K
	MILLIPLEX® MAP Human Neuropeptide Magnetic Bead Panel	HNPMAG-35K
	MILLIPLEX® MAP Human Pituitary Magnetic Bead Panel 1	HPTP1MAG-66K
	MILLIPLEX® MAP Human Pituitary Magnetic Bead Panel 2	HPTP2MAG-66K
	MILLIPLEX® MAP Non-Human Primate Pituitary Magnetic Bead Panel 1	NHPPT1MG-46K
	MILLIPLEX® MAP Non-Human Primate Pituitary Magnetic Bead Panel 2	NHPPT2MG-46K
	Rat/Mouse Neuropeptide Y (NPY) ELISA	EZRMNPY-27K
	Rat/Mouse PYY RIA	RMPYY-68HK

For a complete selection, visit: www.merckmillipore.com

TECHNOLOGY HIGHLIGHT

MILLIPLEX® MAP Multiplexed Detection

Bring your biomarkers to life with assays that give you confidence.

Rely on the quality we build in to each assay kit to product results you trust. In addition to the assay specifications listed in the protocol, we evaluate other performance criteria during our validation process: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

Each MILLIPLEX® MAP kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

Intracellular MILLIPLEX® MAP panels and kits include:

- Stimulated and unstimulated cell lysates provided to qualify assay performance
- Comparison of lysate lots to a reference lot to ensure lot-to-lot consistency
- Detection antibody cocktails designed to yield consistent analyte profiles within panel
- MILLIPLEX® MAP, built on trusted Luminex® technology, offers the broadest selection of analytes across a wide range of disease states and species

Custom Assay Development

Need to develop a specific, sensitive, analytically validated assay for your laboratory? Want to combine analytes from our existing portfolio to design a large-plex screening assay? We develop reliable, custom multiplexed assays (using Luminex® xMAP® technology), as well as single detection (ELISAs, GyroMark™ HT, Singulex® Single Molecule Counting and RIAs) assays for protein research, providing you with:

- Reagents (immunogen design and antibody development)
- Assay development
- Manufacturing (commercial kits for research use only)

Contact your Protein Specialist or e-mail us at: customassay@merckmillipore.com today!

Biomarker Wish List

Is there an analyte, panel or species you would like to see in our portfolio?

Make your wish at:

biomarkerwish@merckmillipore.com

For details, visit: **www.merckmillipore.com/milliplex**

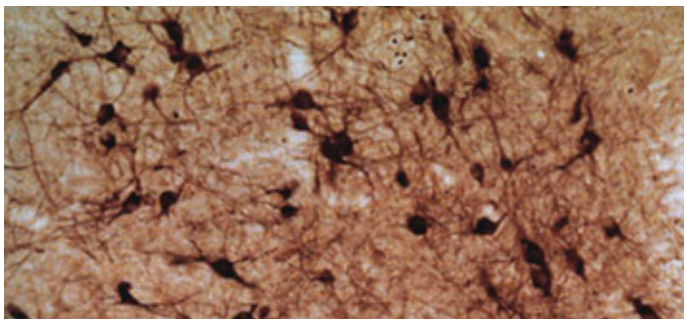
Notes

Oxidative Stress

Production of excessive amounts of reactive oxygen species (ROS), which cause oxidative damage to proteins, lipids, and DNA, plays a key role in a number of pathological disorders.

An excess of free radicals causes oxidative stress, an unstable cellular environment that can result from exposure to alcohol, medications, poor nutrition, trauma, cold, toxins, and over-exercise. Reactive oxygen and nitrogen species (ROS/RNS) and other free radicals also form when cells encounter oxidizing agents or ionizing radiation. ROS and RNS can damage DNA, an early step in carcinogenesis; damage to other biomolecules leads to atherosclerosis, ischemia-reperfusion injury, rheumatoid arthritis, inflammation, diabetes, aging, neurodegenerative diseases, and other disorders.

A major direction in oxidative stress research is focused around the role of ROS/RNS in redox signaling and understanding the mechanisms that disrupt normal redox processes. A major challenge for scientists studying redox signaling continues to be that redox signaling is an essential part of normal development and homeostasis, hence, any experimental and or therapeutic design must be carefully constructed to reduce pathological redox signaling while allowing normal physiological redox signals to continue. Merck Millipore is a pioneer in developing numerous oxidative stress related technologies including carbonyl detection assays, TUNEL apoptosis assays, and benchtop and imaging flow cytometry platforms and assays.



Staining of rabbit brain stem neurons and fibers using Anti-Nitric Oxide Synthase (Catalog Number AB1529).

Did You Know?

A plethora of research has shown that people who eat more vegetables and fruits have lower risks of several diseases; however, it is not clear whether these results are related to the amount of antioxidants in vegetables and fruits, other components in these foods, other factors in people's diets, or lifestyle choices. Rigorous scientific studies involving more than 100,000 people combined have tested whether antioxidant supplements can help prevent chronic diseases, such as cardiovascular diseases, cancer, and cataracts. In most instances, antioxidants did not reduce the risks of developing these diseases.

New directions in oxidative stress research

Imaging oxidative stress: van der Heijden J, Bosman ES, Reynolds LA, Finlay BB. Direct measurement of oxidative and nitrosative stress dynamics in Salmonella inside macrophages. Proc. Natl. Acad. Sci USA. 2015; 112: 560-565.

Oxidative stress in autoinflammatory disease: Lavieri R, Rubartelli A, Carta S. Redox stress unbalances the inflammatory cytokine network: role in autoinflammatory patients and healthy subjects. J Leukoc Biol. 2015; Epub pii: jlb.3MR0415-159R.

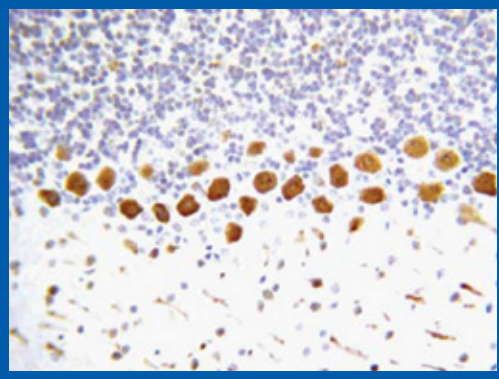
Energy metabolism in neural development: Xavier JM, Rodrigues CMP, Solá S. Mitochondria: Major Regulators of Neural Development. Neuroscientist. 2015; Epub 10.1177/1073858415585472.

ANTIBODY SPOTLIGHT

Nitrotyrosine Detection

The cellular production of highly reactive nitrogen species derived from nitric oxide leads to the nitration of tyrosine resides in proteins. Nitrotyrosine can be detected, estimated semi-quantitatively, and located in cells and tissues by immunocytochemical techniques using antibodies directed against the nitrotyrosine hapten.

Merck Millipore's Anti-Nitrotyrosine antibodies are specific and validated in applications such as IHC, WB, IP, and FC to enable your oxidative stress research.



Staining of normal cerebellum tissue using an Anti-Nitrotyrosine Antibody (Catalog No. AB5411). Nitrotyrosine presence is demonstrated via immunoreactivity in the Purkinje Cells.

Advances in Technology: redox-sensitive GFP probes and imaging flow cytometry

Oxidative stress is dynamic and variable across populations of cells. New bioprobes such as redox-sensitive GFP constructs and autophagic/apoptotic reporters are now becoming available. Concurrently, imaging flow cytometry has made significant advances such that thousands of cells can be imaged in seconds to analyze levels and localization of oxidative stress in heterogeneous eurkaryotic and prokaryotic populations. In combination with these powerful probes, stains, or antibody labels, the ImageStream®^{X} Mark II Imaging Flow Cytometer platform combines the power of digital fluorescence microscopy with the speed and sensitivity and scalability of flow cytometry to enable researchers to approach oxidative stress questions and examine experimental paradigms that are simply not possible with either technique alone.

www.merckmillipore.com/amnis

van der Heijden J, Bosman ES, Reynolds LA, Finlay, BB. Proc. Natl. Acad. Sci USA. 2015; 112: 560 - 565.

Technical Tip

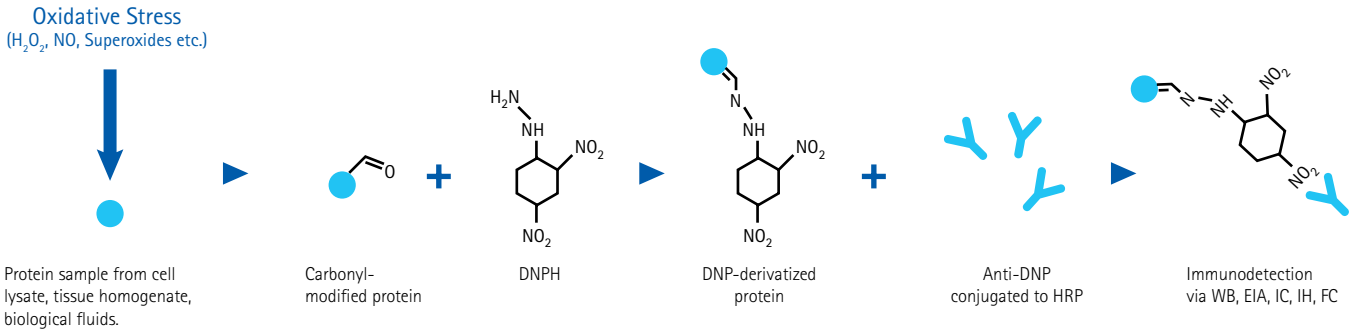
The effects of oxidative stress can be measured on multiple targets not just protein oxidation or mitochondrial potential. Antibodies and probes against modified biomarkers for specific protein modifications (e.g. AGE), lipid peroxidation (e.g., 4-HNE), DNA oxidation (e.g. 8-OHG), or microRNAs (e.g. miR-34).

Featured Technique:

Carbonyl Modification Detection

Oxidative stress has been found to play a key role in a number of pathological disorders. These affects appear to be mediated by reactive oxygen species (ROS) which cause oxidative deterioration of proteins, lipids, and DNA. When it comes to proteins, ROS affect their enzymatic and biochemical functionality, primarily due to the formation of carbonyl derivatives on amino acid side chains. Not surprisingly, carbonyl formation has become an important biomarker for oxidative stress. Merck Millipore's oxidative stress detection kits enable simple and sensitive immunodetection of these carbonyl groups.

The test method involves chemical derivatization of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH). This chemical reaction results in proteins being covalently coupled to DNP at their carbonyl sites. The DNP-derivatized proteins are then detected using an antibody that specifically binds to the DNP moiety. Subsequent incubation with a conjugated secondary antibody and colorimetric development or fluorescence allows detection of protein oxidation.

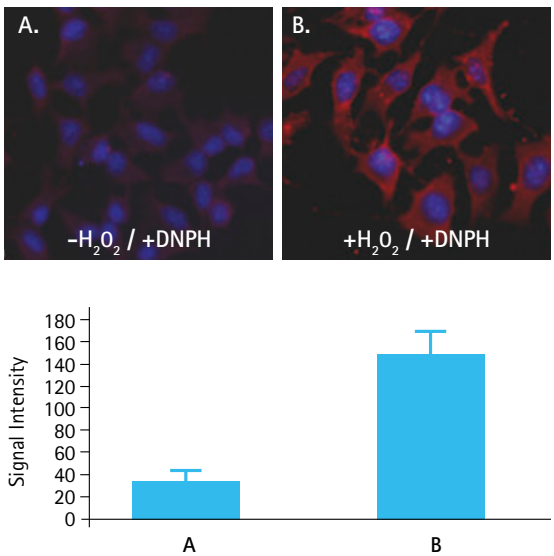


Featured Solution:

OxyICC™ Oxidized Protein Detection Kit

(Catalog No. S7350)

The OxyICC™ kit provides reagents for fluorescent immunocytochemical detection of cellular protein carbonyls. This simple assay detects carbonyl modifications using dinitrophenylhydrazine (DNPH) to provide highly sensitive and quantitative results.



Quantitation of OxyICC™ Analysis. Experiments were performed using HeLa cells and hydrogen peroxide (H_2O_2) treatment (400 µM for 30 minutes). The cells were then analyzed for oxidative stress following the OxyICC™ protocol. For each test reaction the Cy®3 fluorescence pixel intensity was quantified for ten randomly chosen cells. The signals obtained were averaged and then normalized to background to give a signal intensity measurement for each experiment. The data for the three experiments were then averaged and is depicted with error bars. The quantitative measurement shows that the $+H_2O_2$/+DNPH reaction (B) had over a four fold signal intensity increase versus basal levels found in the $-H_2O_2$/+DNPH sample (A). Cell nuclei are blue due to DAPI staining whereas DNP signal is red. Virtually no staining was observed in the control reactions which lack DNPH treatment (not shown).

Solutions for your Research

Merck Millipore offers effective solutions for research on Oxidative Stress:

Research Solutions	Description	Catalog No.
Related Antibodies	Anti-AIF (Apoptosis Inducing Factor)	07-208
	Anti-ATP Synthase, β chain, clone 4.3E8.D1	MAB3494
	Anti-CYP24A1	ABN201
	Anti-CYP27A1	ABC420
	Anti-Cytochrome C, clone EP1326Y, rabbit monoclonal	04-1043
	Anti-Cytochrome P450 (scc), a.a. 509-526	ABS236
	Anti-ETFA	ABS6080
	Anti-Mitochondria	AB3598
	Anti-Mitochondria, clone 113-1, Alexa Fluor® 488 conjugate	MAB1273A4
	Anti-Mitochondria, clone 113-1, Biotin Conjugate	MAB1273B
	Anti-Mitochondria, surface of intact mitochondria, clone 113-1	MAB1273
	Anti-Peroxiredoxin-5 (PRDX5), clone 5 288 2F4	MABN301
	Anti-PITRM1	ABT303
	Anti-PRDX5	ABC281
	Anti-Smac/DIABLO, clone Y12, rabbit monoclonal	04-578
Oxidative Stress Kits & Assays	FlowCellect® Oxidative Stress Characterization Kit	FCCH025111
	FlowCellect® MitoStress Kit	FCCH100109
	Glutathione Detection Kit	APT250
	MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	HOXPSMAG-16K
	MILLIPLEX® MAP Rat/Mouse Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	RMOXPSMAG-17K
	NovaQUANT® Mouse Oxidative Stress qPCR Kit	72628
	OxyBlot™ Protein Oxidation Detection Kit	S7150
	OxyICC™ Oxidized Protein Detection Kit	S7350
	OxyIHC™ Oxidative Stress Detection Kit	S7450
	Superoxide Dismutase Assay Kit II	574061
Reagents, Stains, & Solutions	Chenodeoxycholic Acid, Sodium Salt	220411
	JC-1	420200
	MTT	475989
	Nigericin, Sodium Salt, Streptomyces hygroscopicus	481990

For a complete selection, visit: www.merckmillipore.com

TECHNOLOGY HIGHLIGHT

What if you could have the power of multiparameter cellular analysis at your fingertips?

Innovative

Through a strong heritage and culture of innovation, Merck Millipore has developed the broadest range of flow cytometric analyzers available. The unique capabilities of these cytometers can take your research to the next level.

Amnis® imaging flow cytometers

Amnis® imaging flow cytometers are the first and only cell analyzers to combine the best of flow cytometry and microscopy, yielding novel insights into cell biology and powerfully expanding the range of cell analysis applications.

Muse® cell analyzer

The Muse® cell analyzer packs 3-parameter analysis into a compact, easy-to-use benchtop device, making flow cytometry accessible to anyone, any time.

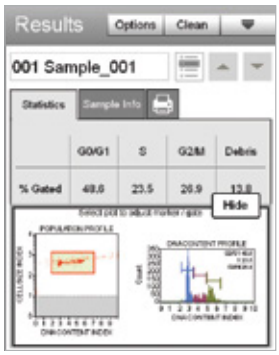
guava easyCyte™ benchtop flow cytometers

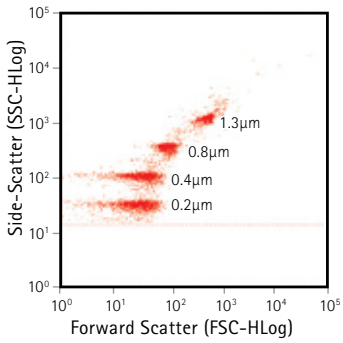
guava easyCyte™ benchtop flow cytometers, featuring patented microcapillary fluidics, consume less sample, generate less waste, and are easier to use and maintain.

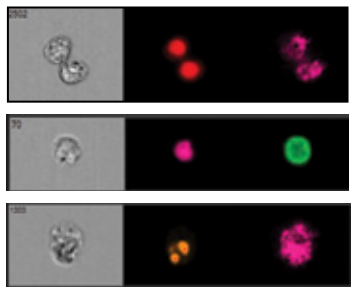
What if anyone in your lab could easily run sophisticated cell health analyses, clearly delineate cell populations, and even visualize and quantify individual cellular events?

Intuitive

Merck Millipore flow cytometry systems are designed for ease of use so you can focus on your research and advance your discovery. Our powerful yet intuitive analysis software is designed to expedite results for common assays, while providing the versatility to perform your assay.







How much more would you accomplish with simple, intuitive assays based on powerful flow cytometry principles?

Flexible

Merck Millipore flow cytometry systems are supported by a broad portfolio of reagents and kits to help you gain greater insight into cellular status and processes. Muse® and FlowCellect® pre-optimized, multi-color kits simplify assessment of cell health, cell signaling, and other applications. The Milli-Mark® line of fluorophore-conjugated antibodies provides an extensive palette for designing custom multi-color reagent combinations.

www.merckmillipore.com/flowcytometry

Through innovation, ease of use, and flexibility, Merck Millipore has created simple, versatile, sensitive, and boundless cytometric analysis platforms.

Pick your platform & Make your mark...

Which instrument is right for you?

		Muse® Cell Analyzer	guava easyCyte™ Flow Cytometer	FlowSight® Imaging Flow Cytometer	ImageStream®X Imaging Flow Cytometer
		Simple	Versatile	Sensitive	Boundless
Function	Lasers	1	1-3	1-4	1-7
	Detection parameters	3	5-12	Up to 12	Up to 12
	Throughput	Single tube	Single tube or 96-well plate	Single tube or 96-well plate	Single tube or 96-well plate
Applications	Image magnification	n/a	n/a	20X	Up to 60X
	Format	Pre-optimized kits	Open	Open	Open
	Flexibility	Low-Med	Med	High	Highest

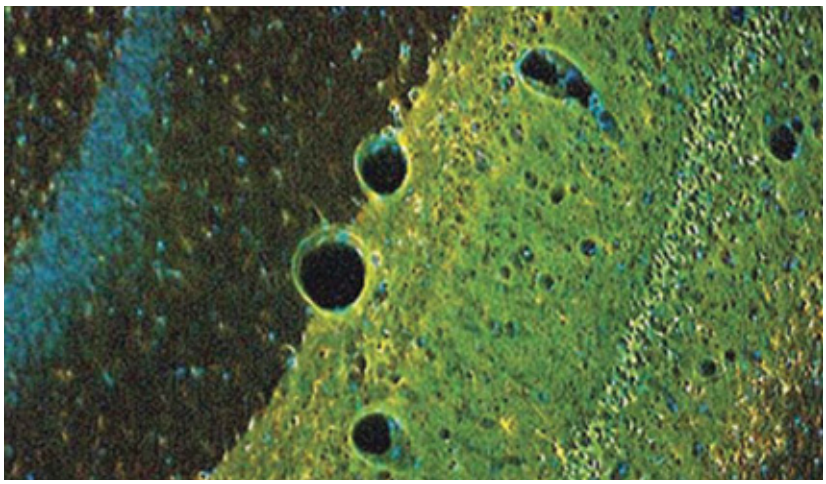
To learn more about cellular analysis technologies or request a demonstration, visit us at: www.merckmillipore.com/cellularanalysis

Notes

Neurodegenerative Disease

Diseases such as Parkinson's, Alzheimer's, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and prion diseases, all follow complex multi-step cellular pathways.

Neurodegenerative diseases are characterized by deterioration of neurons or their myelin sheath, disruption of transmission of sensory information, movement control, and more. Significant advances in early biomarker detection and *in vivo* imaging have aided early assessment of degenerative neurological disease progression. On the mechanistic side, some of the most intriguing recent research into chronic, progressive neurodegenerative disease is focusing on novel molecular pathways. Dysfunction in mitochondrial and autophagic processes, Wnt signaling pathways, and HDAC epigenetic modulatory mechanisms may prove to be key in a number of diseases and therapeutic targeting is underway. Significant attention is also being directed to the role of inflammation in advancing neurodegenerative disease etiology. Merck Millipore offers validated biomarker antibodies, biochemical, and assay platforms for elucidating the pathogenesis of both amyloid- and non-amyloid-related neurodegeneration.



Survey view of triple labeling in the rat hippocampus following exposure to kainic acid. Degenerating neurons stain green with Fluoro-Jade® B, reactive astrocytes stain red with anti-GFAP, and viable cells stain blue with DAPI. Photomicrograph courtesy of Dr. Larry Schmued.

Did You Know?

In the US, every 68 seconds someone is diagnosed with Alzheimer's disease while every 15 seconds someone experiences traumatic brain injury.

New directions in neurodegenerative disease and disorder research

Neuroautophagy: Rubinsztein DC, Bento CF, Deretic V. Therapeutic targeting of autophagy in neurodegenerative and infectious diseases. J. Exp. Med. 2015; 212:979-990.

Epigenetics in disease: Mathias RA, Guise AJ, Cristea IM. Post-translational Modifications Regulate Class IIa Histone Deacetylase (HDAC) Function in Health and Disease. Mol. Cell Proteomics. 2015; 14: 456-470.

Mitochondrial dysfunction: Franco-Iborra S, Vila M, Perier C. The Parkinson Disease Mitochondrial Hypothesis: Where Are We at? Neuroscientist. 2015; Epub 10.1177/1073858415574600.

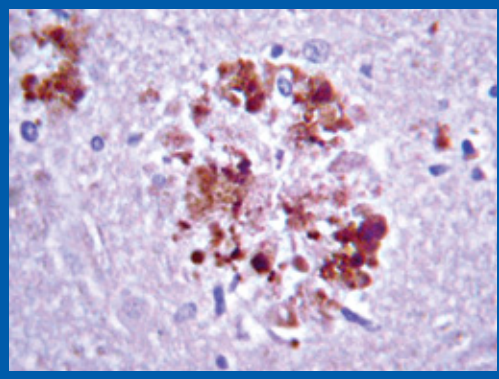
Neuroinflammation: Stojkovska I, Wagner BM, Morrison BE. Parkinson's disease and enhanced inflammatory response. Exp. Biol. Med. 2015; Epub 10.1177/1535370215576313.

ANTIBODY SPOTLIGHT

Alzheimer's Disease: Anti-APP

Deposit of β amyloid peptides near nerve processes are found in the brains of aged human and in cases of Alzheimer's Disease. The principle component of this extracellular amyloid is β A4, a 4 kDa peptide derived from a larger amyloid precursor protein (APP), which is widely expressed in the brain and body. The creation, transport and function of these proteins is currently an active area of research.

This antibody recognizes amino acids 66-81 of the N-terminus on the pre-A4 molecule, and is useful for detecting all three isoforms of APP, immature ~110 kDa, sAPP ~120kDa, and mature ~130 kDa. Also available in various conjugated forms, including biotin, Alexa Fluor® 488, 555, and 647.



Alzheimer's Disease-Hypothalamus tissue was stained using Anti-APP A4 Antibody, a.a. 66-81 of APP (NT), clone 22C11 (Catalog No. MAB348). Immunoreactivity is seen as staining on plaque deposits (dark brown).

Advances in Technology: Precision conformation-specific antibodies

Protein conformations play key roles in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Prion-related diseases, which seem to involve cytotoxic defects in protein folding. Researchers in this area are increasingly choosing conformation-specific antibodies for their structure-function studies, because many of these misfolded protein structures do not withstand X-ray crystallography or solution NMR and are unstable and transient. This specific recognition is possible because an antibody's epitope is usually one to six monosaccharides or 5 to 8 amino acid residues on the surface of the antigen. One can thus develop an antibody that recognizes a specific, three-dimensional antigenic conformation (e.g., a unique site formed by the folding of a linear stretch of amino acids or the interaction of two native protein loops or subunits).

Multiple techniques are now used successfully to create conformation specific antibodies to neurodegeneration related targets.

- Traditional peptide immunogens
- Native/mutant protein immunogens either isolated or recombinant (Stanker et al. 2012)
- Phage display engineered recombinant antibody chains (Paduch et al., 2013)
- Engineered domain fragments, which mimic the conformational binding of the native protein (Habicht et al. 2007)

Learn more at: www.merckmillipore.com search: conformation-specific

Technical Tip

When using knockout and overexpression models in studies of neurodegenerative disease mechanisms, strive to use more comprehensive anatomical and behavioral analyses when studying the effects. APP overexpression models for example, can show far more extensive and wide ranging oxidative stress effects in multiple brain and body regions than just the standard AD plaques that are the typical focus. Understanding the far reaches of genetic manipulations that could influence the disease model will aid interpretation and validate the model system.

Featured Technique:

Biochemicals and Stains

Since the early days of scientific inquiry, when commonly available stains were used to get a better visualization of cellular structures, biochemicals and stains have been indispensable for the study of biology. We have come a long way from Santiago Ramon y Cajal's early drawings of neurons as he observed them. Neuroscience has advanced drastically, but the use of stains and biochemicals remains a very useful tool for researchers even today. Key to understanding the degenerative progression is detecting and staining the dying neurons.

Research essential biochemicals, such as antibiotics, buffers, detergents, dyes, stains, and substrates, are basic components in a wide range of applications. Merck Millipore provides the biochemicals and reagents you need to advance your life science research.

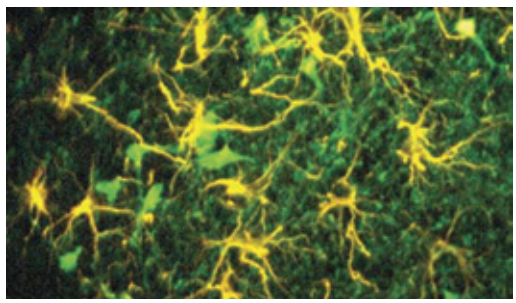
Featured Solution:

Fluoro-Jade® B and Fluoro-Jade® C

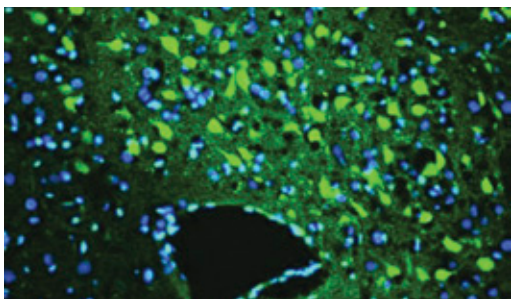
(Catalog Nos. AG310-30MG, AG325-30MG)

The **Fluoro-Jade® dyes** stain all degenerating neurons, regardless of specific insult or mechanism of cell death. Therefore, the patterns of neuronal degeneration seen following exposure to either the glutamate agonist, kainic acid, or the inhibitor of mitochondrial respiration, 3-NPA, were the same for the various Fluoro-Jade® dyes. However, there was a qualitative difference in the staining characteristics of the fluorochromes.

Fluoro-Jade® B is a polyanionic fluorescein derivative which sensitively and specifically binds to degenerating neurons. It is a dark red power that has a green iridescence with excitation peak at 480 nm and emission peak at 525 nm. The filter used for visualizing Fluoro-Jade® B is a fluorescein/FITC filter. Fluoro-Jade® B can be used on most tissue section types and thicknesses including frozen, vibratomed cryostat or paraffin-embedded sections from 3-50 µm. Fluoro-Jade® B is faster and more reliable than older methods (e.g. suppressed silver) for the unequivocal qualitative detection and quantitative measurement of both and fine scale neuronal degeneration.



Double labeling within the hilar region of the rat hippocampus can be seen following exposure to the excitotoxin, kainic acid. Degenerating neurons stain green with Fluoro-Jade® B, while astrocytes appear red following immunofluorescent labeling with anti-GFAP. Photomicrograph courtesy of Dr. Larry Schmued.



Double exposure using ultraviolet and blue light excitation reveals blue DAPI labeled nuclei and green Fluoro-Jade® C (Catalog No. AG325) positive cells and terminals in the dorsal thalamus following kainic acid exposure. Photomicrograph courtesy of Dr. Larry Schmued.

Fluoro-Jade® C also stains all degenerating neurons, but exhibits the greatest signal to background ratio, as well as the highest resolution. This translates to a stain of maximal contrast and affinity for degenerating neurons. Fluoro-Jade® C is ideal for localizing not only degenerating nerve cell bodies, but also distal dendrites, axons and terminals. The dye is highly resistant to fading and is compatible with virtually all histological processing and staining protocols.

Also available:

Description	Catalog No.
Black-Gold® II Myelin Staining Kit	AG105
FLUORO-RUBY®	AG335
Black-Gold® II Stain	AG400

Solutions for your Research

Merck Millipore offers effective solutions for research on Neurodegenerative Disease:

Research Solutions	Description	Catalog No.
Alzheimer's Disease – Antibodies	Anti-Alzheimer Precursor Protein A4, a.a. 66-81 of APP (NT), clone 22C11	MAB348
	Anti-Amyloid Fibril LOC	AB2287
	Anti-Amyloid Fibril OC	AB2286
	Anti-β-amyloid 1-42	AB5078P
	Anti-Human Amyloid β, clone WO-2	MABN10
	Anti-Human Amyloid β40, clone G2-10	MABN11
	Anti-Human Amyloid β42, clone G2-11	MABN12
	Anti-Human Amyloid β42, clone G2-13	MABN13
	Anti-pan amyloid β peptide (MOAB2), clone 6C3	MABN254
	Anti-Tau phospho Threonine 231	AB9668
Alzheimer's Disease – Assays	High Sensitivity Human Amyloid β40 ELISA	EZHS40
	High Sensitivity Human Amyloid β42 ELISA	EZHS42
	High Sensitivity Human Amyloid β40 and Amyloid β42 ELISA	EZHS-SET
	Human Amyloid β40 Brain ELISA	EZBRAIN40
	Human Amyloid β42 Brain ELISA	EZBRAIN42
	Human Amyloid β40 and Amyloid β42 Brain ELISA	EZBRAIN-SET
	Human S100B ELISA	EZHS100B-33K
	MILLIPLEX® MAP Amyloid β and Tau Multiplex Panel	HNABTMAG-68K
	MILLIPLEX® MAP Human Neurodegenerative Disease Magnetic Bead Panel 1	HNDG1MAG-36K
	MILLIPLEX® MAP Human Neurodegenerative Disease Magnetic Bead Panel 2	HNDG2MAG-36K
	MILLIPLEX® MAP Human Neurodegenerative Disease Magnetic Bead Panel 3	HNDG3MAG-36K
	MILLIPLEX® MAP Human Neurodegenerative Disease Magnetic Bead Panel 4	HNDG4MAG-36K
	MILLIPLEX® MAP Human Neurological Disorders Magnetic Bead Panel 1	HND1MAG-39K
Huntington's Disease	Anti-Huntingtin Protein, a.a. 181-810, clone 1HU-4C8	MAB2166
	Anti-Huntingtin Protein, clone mEM48	MAB5374
	Anti-Huntingtin Associated Protein 40 (HAP40)	AB5872
	Polyglutamine-Expansion Diseases Marker	MAB1574
Parkinson's Disease	Anti-Dardarin (LRRK2)	AB9682
	Anti-Dopamine D2 Receptor	AB5084P
	Anti-Parkin	AB9244
	Anti-PINK1 Antibody, clone N4/49	MABN18
	Anti-Synuclein, α	AB5038P
	Anti-Tyrosine Hydroxylase	AB152
	N27 Rat Dopaminergic Neural Cell Line	SCC048

Continued on next page

Merck Millipore offers effective solutions for research on Neurodegenerative Disease:

Research Solutions	Description	Catalog No.
Prion Diseases	Anti-14-3-3 phospho Serine58	AB9750
	Anti-Clusterin, α chain	05-354
	Anti-Prion Protein	AB5058
	Anti-Prion Protein, clone 2G11	MAB5542
	Prion Protein	AG210
Amyotophic Lateral Sclerosis (ALS)	Anti-Calbindin D-28K	AB1778
	Anti-Neuroketal	AB5611
	Anti-p75 NTR	AB1554
	Anti-Superoxide Dismutase 1 (SOD1)	AB5480
Multiple Sclerosis (MS)	Anti-Degraded Myelin	AB5864
	Anti-H-CAM (CD44)	CBL1308
	Anti-MOG	MAB5584
	Black-Gold® II Myelin Staining Kit	AG105
	Human Oligodendrocyte Differentiation Kit	SCR600
General Neuronal Degeneration	Anti-Beclin 1	AB15417
	ChemiKine™ Pigment Epithelium Derived Factor, Sandwich ELISA	CYT420
	FLUORO-Jade® C	AG325
	FLUORO-Ruby®	AG335
	GFAP ELISA	NS830
	Human sICAM-1 ELISA	ECM335
	Human sVCAM-1 ELISA	ECM340
	LentiBrite™ GFP-LC3 lentiviral Biosensors	17-10193
	LentiBrite™ GFP-LC3 Mutant lentiviral Biosensors	17-10189
	LentiBrite™ GFP-PSD95 Lentiviral Biosensors	17-10227
	LentiBrite™ RFP-PSD95 Lentiviral Biosensors	17-10226
	Mouse SAA-3 ELISA	EZMSAA3-12K
	Phosphorylated Neurofilament ELISA	NS170
Inhibitors	β-Amyloid Oligomer Inhibitor, K01-162	200487
	β-Secretase Inhibitor IV	565788
	γ-Secretase Inhibitor IX	565770
	γ-Secretase Inhibitor XX	565789
	γ-Secretase Inhibitor XXI, Compound E	565790
	γ-Secretase Inhibitor XXIV, BMS299897	565793
	InSolution™ γ-Secretase Inhibitor X	565771
	LRRK2-IN-1	438193

For a complete selection, visit: www.merckmillipore.com

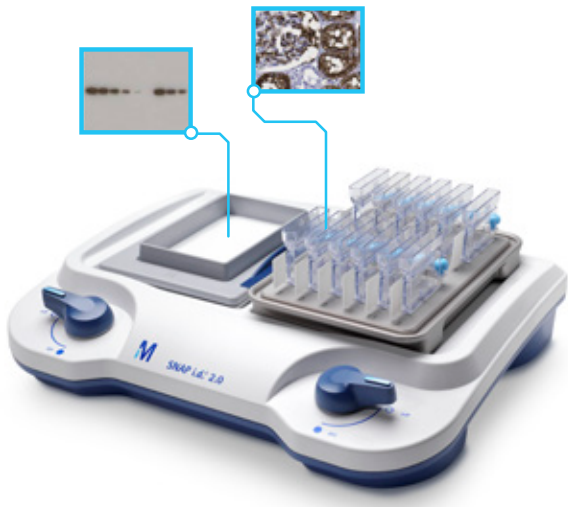
TECHNOLOGY HIGHLIGHT

SNAP i.d.® 2.0 system for Western blotting and IHC. Multiple slides, multiple blots, multiple conditions.

There's so much room for experimental variability in traditional immunodetection workflows. For your peace of mind – and ours – we designed the SNAP i.d.® 2.0 system to streamline your Western blot and immunohistochemistry experiments.

The concept is simple: a vacuum-driven flow of blocking, antibody, and washing solutions reduces slide and membrane handling. That means a lot less shaking, dipping, pouring, and waiting.

And now you can process multiple blots and slides in parallel, so it's easy to apply consistent conditions across experiments.



SNAP i.d.® 2.0 Protein Detection System for Western Blotting

Unlike conventional Western blotting, where diffusion is the primary means of reagent transport, the SNAP i.d.® 2.0 system applies a vacuum to actively drive reagents through the membrane. This advanced technology promotes antigen binding and thorough washing, enabling you to better optimize your Western blotting conditions.

The SNAP i.d.® 2.0 Systems for Western blotting contain everything you need to get started, including the detection base, 2 blot holding frames, 2 blot holders, 2 antibody collection trays, a blot roller and rolling pad, 2 wetting trays, vacuum tubing, and a Quick Start User Guide.

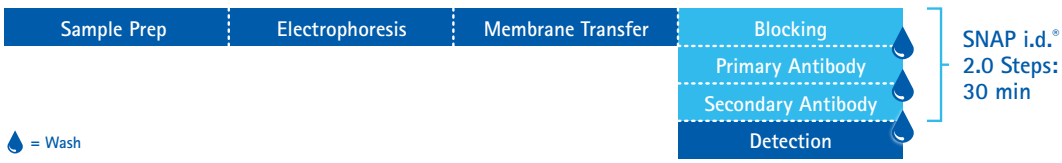
Key Features

- Faster results
- Faster testing of different antibodies
- Higher throughput of Western blots each day

Advantages of the Vacuum Transport Feature

- Draws reagents through blotting membrane
- Minimizes over-blocking
- Thoroughly flushes membranes instead of just rinsing
- Reduces incubation times

SNAP i.d.® 2.0 system in the Western blotting workflow



Faster Blots, Better Signals. Comparison of the traditional Western blotting protocol relative to SNAP i.d.® 2.0 system's 30 minute protocol.

Take protein detection to new dimensions: www.merckmillipore.com/SNAP

SNAP i.d.® 2.0 IHC System

SNAP i.d.® 2.0 Protein Detection System for Immunohistochemistry (IHC) introduces a new capability to the innovative, vacuum-driven SNAP i.d.® 2.0 system. The IHC frame and slide holders allow you to block, probe, and stain up to 12 tissue slides per frame. Reduced handling time and multiple-slide processing make this system ideal for antibody and protocol optimization.

The SNAP i.d.® 2.0 Systems for IHC contain everything you need to get started, including the detection base, IHC frame and incubation cover, slide holders, an assembly fixture, vacuum tubing and a Quick Start User Guide.

Key Features

- Flexibility of multiple slide configurations enables the processing of 1 to 24 slides at a time
- Compatible with standard IHC slides and protocols
- Compatible with diverse tissue preparations including formalin-fixed or fresh frozen samples
- Intuitive format:
  - Incorporates blocking, washing, and antibody incubation and labeling steps
  - Systematizes handling multiples slides without the cost of automation
- Test tracker feature on frame cover helps keep track of IHC steps

Key Benefits

- Eliminates the need for pap pens
- Antibodies can be collected and reused
- Slide handling time is significantly decreased
- Less time spent on wash steps
- Parallel processing of multiple slides

Immunohistochemistry workflow includes blocking, antibody incubations, labeling and wash steps, all of which can be streamlined using the SNAP i.d.® 2.0 Protein Detection System for IHC.

Description	Catalog No.
SNAP i.d.® 2.0 System - Mini (7.5 x 8.4 cm)	SNAP2MINI
SNAP i.d.® 2.0 System - Midi (8.5 x 13.5 cm)	SNAP2MIDI
SNAP i.d.® 2.0 System - MultiBlot (4.5 x 8.4 cm)	SNAP2MB3
SNAP i.d.® 2.0 Protein Detection System - Single IHC	SNAP2IHC
SNAP i.d.® 2.0 Protein Detection System - Double IHC	SNAP2IHC2

Notes

Neuroscience

Research Solutions Guide

What's inside...

Eight chapters on key Neuroscience topics, each with:

- An Introduction
- New Directions
- Featured Technique
- Featured Solution
- Effective Solutions for your Research
- Technology Highlight

And more...

- Did You Know? (informational bits)
- Antibody Spotlight
- Advances in Technology
- Technical Tip (for research)

Discover Effective Solutions Potentiate Your Research on:

- Synapses, Neurons, and Glia
- Neural Stem Cell Markers
- Neurotrophic Factors
- Neurite Outgrowth
- Transmitters & Receptors
- Ion Channels & Transporters
- Sensory Systems and Control
- Oxidative Stress
- Neurodegenerative Disease

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