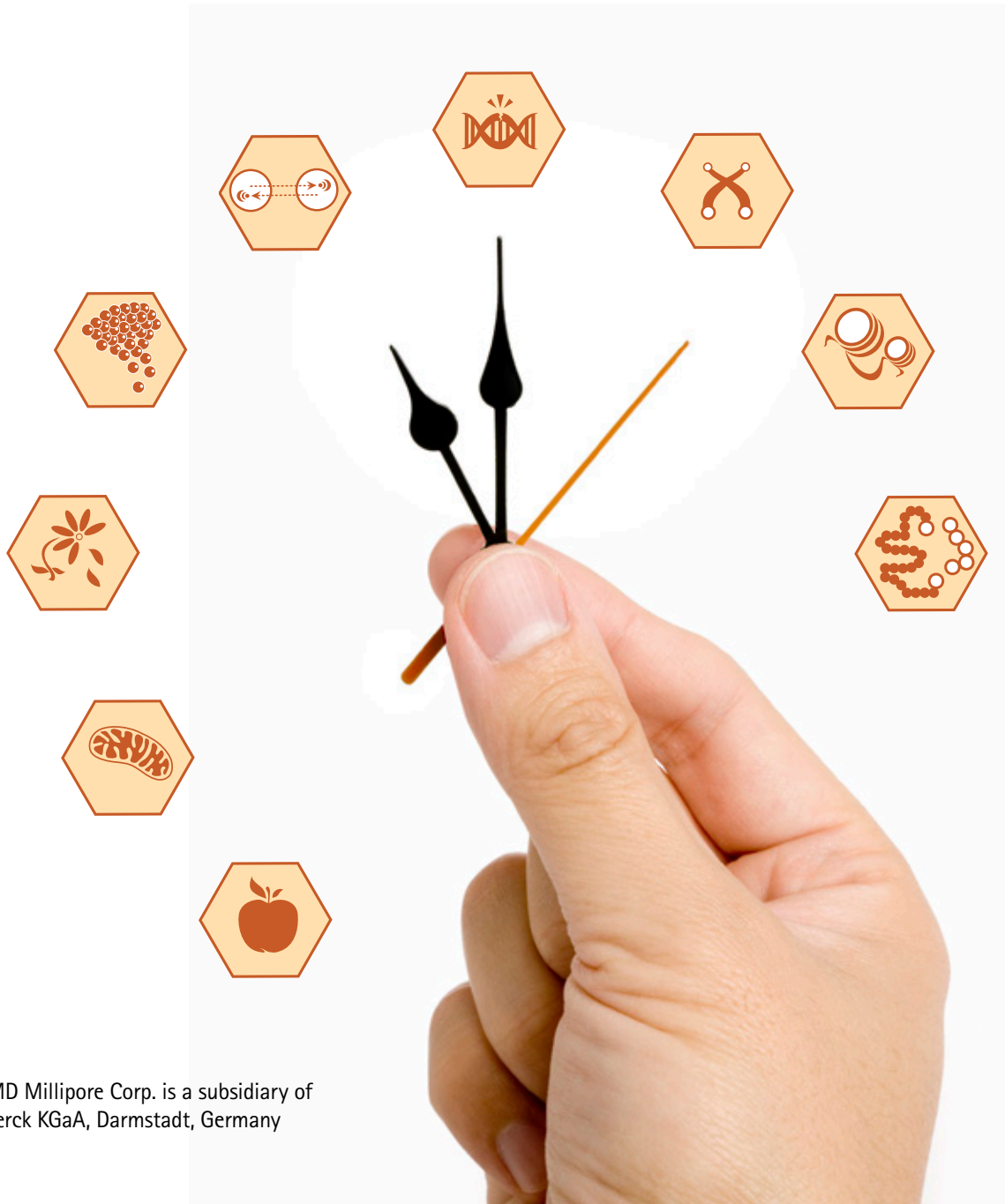
Hallmarks of Aging

Solutions for life science research



EMD Millipore Corp. is a subsidiary of
Merck KGaA, Darmstadt, Germany

Introduction

Aging: getting older, exhibiting the signs of age, the decline in the physical (and mental) well-being over time, leading to death. Since the beginning of time, man has been obsessed with trying to slow down, stop, or even reverse the signs of aging. Many have gone as far as experimenting with nutritional regimens, eccentric exercises, fantastic rituals, and naturally occurring or synthetic wonder-elements to evade the signs of normal aging. Biologically speaking, what is aging? And what does the latest research tell us about the possibility of discovering the elusive "fountain of youth"? Many advances in our understanding of aging have come from systematic scientific research, and perhaps it holds the key to immortality.

Scientifically, aging can be defined as a systems-wide decline in organismal function that occurs over time. This decline occurs as a result of numerous events in the organism, and these events can be classified into nine "hallmarks" of aging, as proposed by López-Otin et al. (2013). Several of the pathologies associated with aging are a direct result of these events going to extremes and may also involve aberrant activation of proliferation signals or hyperactivity.

The hallmarks of aging have been defined based on their fulfillment of specific aging related criteria, such as manifestation during normal aging, acceleration of aging if experimentally induced or aggravated, and retardation of aging if prevented or blocked, resulting in increased lifespan. The nine hallmarks of aging are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

The biological processes underlying aging are complex. By understanding the hallmarks in greater detail, we can get closer to developing intervention strategies that can make the aging process less of a decline, and more of a recline.

In this solutions guide, you will find key concepts and latest findings related to the hallmarks of aging, and also discover EMD Millipore's solutions for the investigation of the processes associated with the various hallmarks. In addition, you will learn some interesting facts about the hallmarks and, we hope, find inspiration to carry on your aging related life science research... gracefully.

Reference: López-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153(60):1194–1217.

These hallmarks are not always independent, and the occurrence of one can, and often does, impact others. For instance, changes in the epigenetic regulation of genes can result in downstream effects on cellular function, protein stability, cell signaling etc. For this reason, the hallmarks can be categorized into three groups:

- **Primary hallmarks (blue icons)** are those that are the foundational causes of cellular damage
- **Response or compensatory hallmarks (orange icons),** which are a result of the primary hallmarks
- **Integrative hallmarks (green icons)** that incorporate the first two classes of hallmarks and ultimately lead to the functional decline observed in aging

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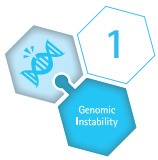
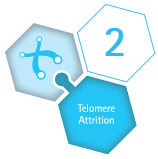
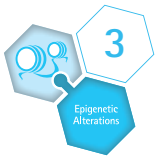
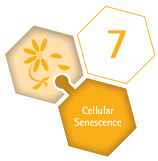
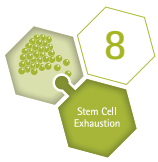


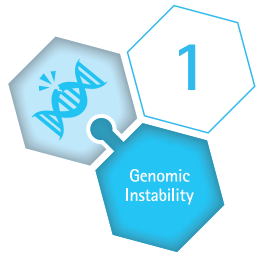








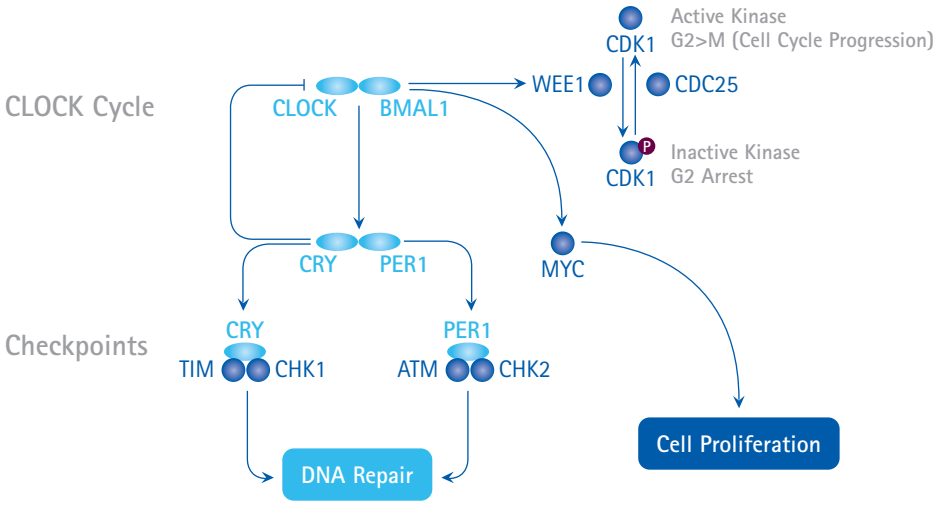
Genomic Instability



As cells age, their chromosomes become less stable. As repair mechanisms fail to correct DNA damage, mutations accumulate and lead to aging and disease. Genomic instability is a key hallmark of aging.

Although there is much evidence linking genomic and epigenomic changes to disorders involving accelerated aging, it's been trickier to relate these changes to natural aging. Of all the DNA lesions that occur as a result of endogenous and environmental insults, it's clear that some (like retrotransposon movement and large chromosomal rearrangements) contribute more than others.

Underlying all the manifestations of genomic instability is compromised DNA repair. Indeed, today's aging research focuses on age-related changes in activation of key DNA repair pathways, involving p53, ATM, histone H2A.X, XRCC4, and ligase 4. What environmental factors can hinder DNA repair? Evidence points to general physiological stress (exacerbated by behaviors like chronic alcohol consumption) and disrupted circadian rhythm. That's right – don't stay up all night, so your genome can recover from daytime damage.



Did you know?

The human genome contains 3 billion base pairs of DNA. DNA damage occurs at a rate of 10,000 to 1,000,000 molecular lesions per cell per day. Though only a small fraction, unrepaired lesions accumulate with age and add to increased incidence of diseases such as cancer.

Of course, it's also possible that decreased DNA repair is itself a symptom, not a cause, of aging. To investigate this possibility, researchers will need to examine changes in transcriptional regulators of repair genes, such as ncRNAs, hormones, and other chromatin modulators.

New Publications

- Walter D, Lier A, Geiselhart A, Thalheimer FB, Huntscha S, Sobotta MC, Moehrle B, Brocks D, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015; 520(7548):549-52.
- Kalfalah F, Seggewiß S, Walter R, Tigges J, Moreno-Villanueva M, Bürkle A, Ohse S, Busch H, et al. Structural chromosome abnormalities, increased DNA strand breaks and DNA strand break repair deficiency in dermal fibroblasts from old female human donors. Aging (Albany NY). 2015; 7(2):110-22.
- Belancio VP, Blask DE, Deininger P, Hill SM, Jazwinski SM. The aging clock and circadian control of metabolism and genome stability. Front Genet. 2015;5:455.

We are a lot like our cells...

Just like our cells suffer from genomic instability, as we age, we get wobbly too. We buy better shoes. Our cells phosphorylate their histone H2A.X.

The Hallmark & Disease

Werner Syndrome (WS), or adult progeria, has an incidence rate of less than 1 in 100,000 live births. WS is caused by mutation in the WRN gene which is responsible for the maintenance and repair of DNA. Patients with Werner Syndrome often age prematurely and develop appearance and features associated with normal aging.

Did you know?

Protein coding genes make up only 3 percent of your DNA. The other 97 percent, the noncoding DNA, controls the activity of your genes. It has recently become apparent that the safeguarding of DNA integrity depends on small ncRNAs acting at the site of DNA lesions to signal the presence of DNA damage in the cell, and on the genes involved in their biogenesis to achieve accurate DNA repair.

Genomic Instability Detection

Featured Technique:

TUNEL Assay

DNA fragmentation is usually associated with ultrastructural changes in cellular morphology in apoptosis. The ApopTag™ family of kits examines apoptosis via DNA fragmentation by the TUNEL assay. The DNA strand breaks are detected by enzymatically labeling the free 3'-OH termini with modified nucleotides. These new DNA ends that are generated upon DNA fragmentation are typically localized in morphologically identifiable nuclei and apoptotic bodies. In contrast, normal or proliferative nuclei, which have relatively insignificant numbers of DNA 3'-OH ends, usually do not stain with the kit. ApopTag™ Kits detect single-stranded and double-stranded breaks associated with apoptosis. Drug-induced DNA damage is not identified by the TUNEL assay unless it is coupled to the apoptotic response. In addition, this technique can detect early-stage apoptosis in systems where chromatin condensation has begun and strand breaks are fewer, even before the nucleus undergoes major morphological changes.

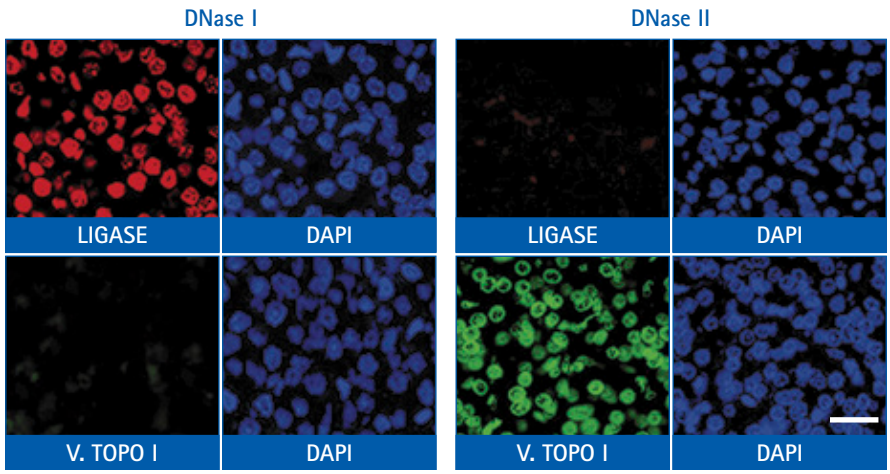
While conventional in situ detection techniques such as ISEL (Klenow DNA polymerase), TUNEL (terminal deoxynucleotidyl transferase, TdT) and ISNT (DNA Polymerase I) are useful in detecting internucleosomal DNA cleavage, they do not differentiate DNase Type I and DNase Type II cleavage. The ISOL technique shows concordant results with the TUNEL technique in specimens without necrosis, and in specimens presenting necrosis, the ISOL technique shows improved selectivity as compared to TUNEL.

Featured Solution:

ApopTag™ ISOL Dual Fluorescence Apoptosis Detection Kit (DNase Types I & II)

(Catalogue No. APT1000)

During apoptosis, one class of DNases, which includes DNase I, is activated via caspase signaling, while the other class, which includes DNase II, is activated via a caspase independent pathway. The ApopTag™ ISOL Apoptosis Kit facilitates the differentiation of apoptotic cells from necrotic or transiently damaged cells by using a proprietary dual fluorescent-labeled oligonucleotide to detect simultaneously both DNase I and DNase II-type DNA fragments in a single sample, thus distinguishing caspase-dependent and caspase–independent apoptotic events.



Selective detection of two major types of DNA damage. Sections of normal bovine adrenal tissue were treated with either DNase I to produce 3'OH/5'PO4 blunt-ended breaks or DNase II to produce 3' PO4/5' OH blunt-ended breaks. Bar - 25 µm.

Solutions for your Research

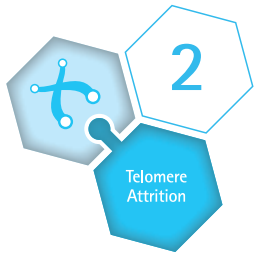
EMD Millipore offers effective solutions for research on topics related to Genomic Instability:

Research	Description	Cat. No.
Apoptosis Detection	ApopTag™ ISOL Dual Fluorescence Apoptosis Detection Kit (DNase Types I & II)	APT1000
	ApopTag™ Peroxidase *In Situ* Apoptosis Detection Kit	S7100
	ApopTag™ Plus Peroxidase *In Situ* Apoptosis Kit	S7101
	ApopTag™ Fluorescein *In Situ* Apoptosis Detection Kit	S7110
	ApopTag™ Plus *In Situ* Apoptosis Fluorescein Detection Kit	S7111
	ApopTag™ Fluorescein Direct *In Situ* Apoptosis Detection Kit	S7160
	ApopTag™ Red *In Situ* Apoptosis Detection Kit	S7165
	ApopTag™ Peroxidase *In Situ* Oligo Ligation (ISOL) Kit	S7200
	Caspase 3 Colorimetric Activity Assay Kit, DEVD	APT165
	Caspase 8 Colorimetric Activity Assay Kit, IETD	APT171
	Caspase 9 Colorimetric Activity Assay Kit, LEHD	APT173
	CaspaTag™ Pan-Caspase *In Situ* Assay Kit, Fluorescein	APT400
	CaspaTag™ Caspase 3,7 *In Situ* Assay Kit, Fluorescein	APT403
	Apo-Direct TUNEL Assay Kit	APT110
DNA Fragmentation	FragEL DNA Fragmentation Detection Kit, Colorimetric - Klenow Enzyme	QIA21
	FragEL DNA Fragmentation Detection Kit, Colorimetric - TdT Enzyme	QIA33
	FragEL DNA Fragmentation Detection Kit, Fluorescent - TdT Enzyme	QIA39
Histone Modification	H2A.X Phosphorylation Assay Kit (Flow Cytometry)	17-344
	H2A.X Phosphorylation Assay Kit (Chemiluminescence Detection)	17-327
	FlowCellect® DNA Damage Histone H2A.X Dual Detection Kit	FCCS025153
	Muse® H2A.X Activation Dual Detection Kit	MCH200101
	FlowCellect® Histone H2A.X Phosphorylation Assay Kit	FCCS100182
	FlowCellect® Multi-Color DNA Damage Response Kit	FCCH025104
	Anti-Histone H2A.X Antibody	07-627
	Anti-Ubiquityl Histone H2A.X (Lys119) Antibody	AB10029
	Anti-phospho-Histone H2A.X (Ser139) Antibody, clone JBW301	05-636

For a complete selection, visit: www.emdmillipore.com/apoptosis

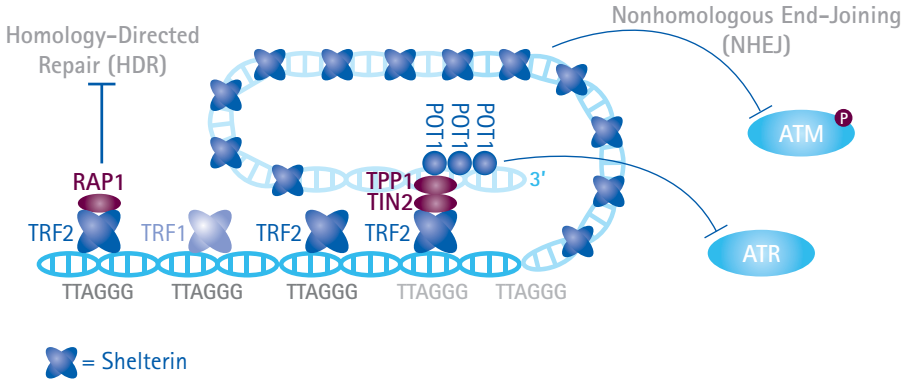
Notes

Telomere Attrition



As cells divide, the telomere ends of chromosomes get shorter. Eventually, the enzyme that adds telomeric repeat sequences, telomerase, gets silenced and the telomeres are too short for cells to divide.

Telomeres at the ends of chromosomes, like all other sections of DNA, are prone to DNA damage, including double-strand breaks (DSBs). And unlike the rest of the chromosome, telomere DSBs aren't fixed by the DNA repair pathway, as this would frequently lead to fused chromosomes and genomic instability. That's why we have telomerase. However, telomerase expression is silenced in many adult cells, to curb rampant cell proliferation and tumorigenesis, and so telomeres get progressively shorter with age.



Telomeres are bound by protein complexes of TRF2, shelterin, and RAP1 to inhibit homology-directed repair (HDR). The T-loop structure at telomere ends, also containing TIN2, TPP1, and POT1, blocks DSB repair.

Did you know?

In humans, the telomere sequence is TTAGGG, usually repeated about 3,000 times. Telomeres can reach up to 15,000 base pairs in length.

After all, most relevant vertebrate models have life spans that are at least 3 years....and who wants to be in graduate school for 6 full generations of mice? Hence, the killifish model of aging*, a vertebrate with a 6-month lifespan with a CRISPR-Cas9-editable genome, is poised to dramatically accelerate telomere attrition research.

New Publications

- *Harel I, Benayoun BA, Machado B, Singh PP, Hu CK, Pech MF, Valenzano DR, Zhang E, et al. A platform for rapid exploration of aging and diseases in a naturally short-lived vertebrate. Cell. 2015;160(5):1013-26.
- Wang J, Zhao C, Zhao A, Li M, Ren J, Qu X. New insights in amyloid Beta interactions with human telomerase. J Am Chem Soc. 2015;137(3):1213-9.
- Borah S, Xi L, Zaug AJ, Powell NM, Dancik GM, Cohen SB, Costello JC, Theodorescu D, Cech TR. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science. 2015 Feb;347(6225):1006-10.

We are a lot like our cells...

Just like the telomere ends of chromosomes in our cells get shorter over time, as we age, we get shorter too. We hem our pants. Our cells enter replicative senescence.

The Hallmark & Disease

Cancer, characterized by the rapid and uncontrolled division of cells, may be the result of abnormally high levels of telomerase activity, which counters telomere attrition. The cancer cells can, therefore, continue to divide and become immortal. Because of this phenomenon telomeres and telomerase have been attractive targets for anticancer drug development.

Did you know?

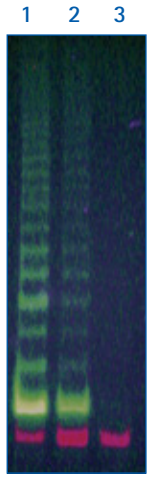
Telomere activity is controlled by two opposing mechanisms. Each time a cell divides, telomeres undergo shortening or erosion. Telomerase enzymes carry out addition to help to build up telomeres. Each time a cell divides, 25-200 bases are lost from the ends of the telomeres on each chromosome. Telomerase has been detected in and found to be 10-20 times more active in human cancer cells than in normal body cells. Research on telomeres and telomerases may lead to information on how to slow down aging and fight cancer.

Telomerase Assay

Featured Technique:

TRAP Assay

The development of a sensitive and efficient PCR-based telomerase activity detection method, TRAP (Telomeric Repeat Amplification Protocol), has made possible large scale surveys of telomerase activity in human cells and tissues. To date, telomerase activity has been detected in over 85% of all tumors tested spanning more than 20 different types of cancers. EMD Millipore's TRAPeze® Telomerase Detection Kits are highly sensitive *in vitro* assay systems for detecting telomerase activity using an improved version of the TRAP method.



EMD Millipore provides a broad range of products for assaying telomerase activity. TRAPeze® telomerase detection kits are rapid, quantitative, *in vitro* assays for detecting activity. The original kit permits detection via PCR and gel electrophoresis. TRAPeze® telomerase detection kits are also available in colorimetric and fluorimetric formats as the TRAPeze® ELISA, Trapeze RT (Real-Time) and TRAPeze® XL kits, incorporating biotinylated and fluorescent primers, respectively.

Image (right) demonstrates the direct fluorescence imaging of the TRAPeze® XL reaction of three specimens – telomerase positive (lanes 1 and 2) and telomerase negative (lane 3).

Featured Solution:

TRAPeze® Telomerase Detection Kit

(Catalogue No. S7700)

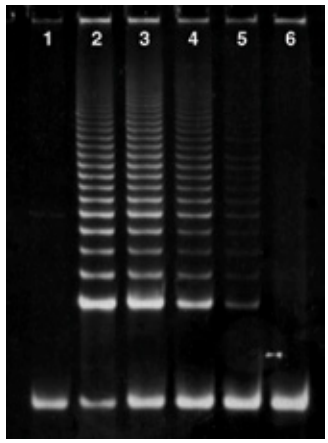
The assay is a one buffer, two enzyme system utilizing the polymerase chain reaction (PCR). In the first step of the reaction, telomerase adds a number of telomeric repeats (GGTTAG) onto the 3' end of a substrate oligonucleotide (TS). In the second step, the extended products are amplified by PCR using the TS and RP (reverse) primers, generating a ladder of products with 6 base increments starting at 50 nucleotides: 50, 56, 62, 68, etc.

The assay enhances the sensitivity of telomerase detection in small samples.

The TRAPeze® Gel-Based Telomerase Detection Kit provides substantial improvements to the original TRAP assay, such as a modified reverse primer sequence which:

- Eliminates the need for a wax barrier hot start,
- Reduces amplification artifacts and
- Permits better estimation of telomerase processivity.

Incorporation of internal positive control makes it possible to quantitate telomerase activity more accurately (with a linear range close to 2.5 logs) and to identify false-negative samples that contain Taq polymerase inhibitors.



Serially diluted extracts of telomerase-positive control cells (provided in the TRAPeze® Kit) were subjected to the TRAP assay. Reaction products were run on a native polyacrylamide gel and stained with SYBR® Green. Lane 1: CHAPS Lysis Buffer control; Lanes 2-5: extract from 10,000, 2000, 400 and 80 cells; Lane 6: heat-treated extract from 10,000 cells.

Solutions for your Research

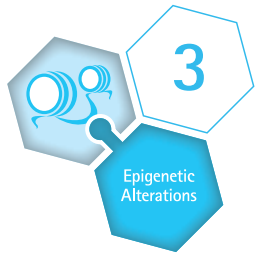
EMD Millipore offers effective solutions for research on topics related to Telomeres and Telomerases:

Research	Description	Cat. No.
Telomerase Detection	TRAPeze® Telomerase Detection Kit (S7700)	S7700
	TRAPeze® XL Telomerase Detection Kit (S7707)	S7707
	TRAPeze® RT Telomerase Detection Kit (S7710)	S7710
Related Small Molecules	Telomerase Inhibitor III, Sodium Salt	581004
	Telomerase Inhibitor IX	581011
	Telomerase Inhibitor X, BIBR1532	508839
	TMPyP4	613560
	PIPER	528120
Related Antibodies	Anti-Telomerase (Ab-1) Rabbit pAb	582000
	Anti-Telomerase (Ab-2) Rabbit pAb	582005
	Anti-hTERT (CT) Antibody, clone Y182, rabbit monoclonal	MABE14
	Anti-TERT (human) Antibody, clone 2C4	MABD55
	Anti-TRF1 Antibody, clone BED5 57-6	04-638
	Anti-TRF1 Antibody	ABE210
	Anti-phospho TRF2 (Thr188) Antibody	07-737
	Anti-TRF2 Antibody, clone 4A794	05-521
	Anti-phospho TIN2 (Ser295) Antibody	ABE1314
	Anti-RAP1 Antibody, clone 4C8/1	05-911
	Anti-Rap1 Antibody	07-916
	Anti-TPP1 Antibody	ABE693
	Anti-ATM Antibody	07-1286
	Anti-ATM Antibody, clone Y170, rabbit monoclonal	04-200
	Anti-phospho-ATM (Ser1981) Antibody, clone 10H11.E12	05-740
	Anti-phospho-ATM (Ser1981) Antibody, clone 10H11.E12 PE conjugate	FCMAB10P

For a complete selection, visit: www.emdmillipore.com/epigenetics

Notes

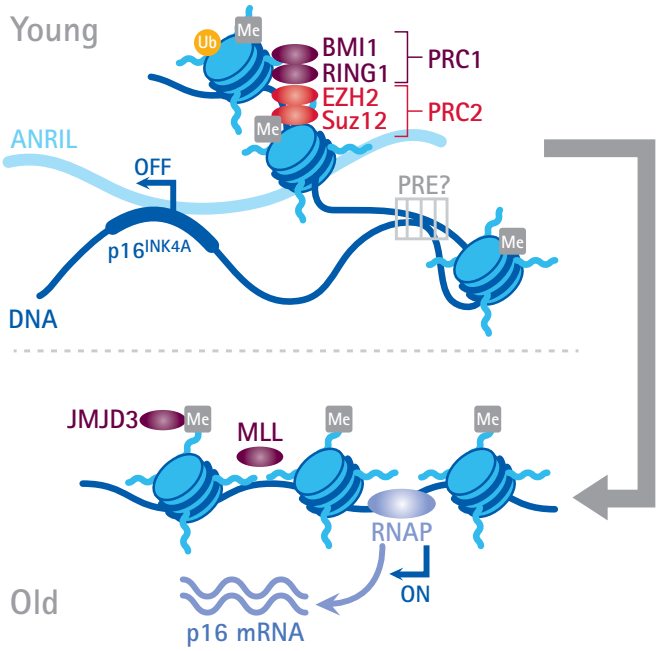
Epigenetic Alteration



As cells are exposed to environmental factors, they are subject to changes in their genome through epigenetic mechanisms. Such changes accumulate over time and have been correlated with the decline observed in aging cells.

Epigenetic changes in aging include decreased methylation of H3K9 and H3K27, together with increased trimethylation of H4K20 and H3K4. In general, these changes are correlated with decreases in the amount of heterochromatin and consequent increases in chromosome fragility and transcriptional noise.

Why do these molecular events occur? One theory is that age-associated DNA damage results in changes in the transcription of certain long noncoding RNAs (lncRNAs), such as KCNQ1OT1, PINT, and ANRIL, which then, in turn, regulate histone modifying enzymes. Whether this mechanism is directly relevant to aging remains to be proven.



Epigenetic modulators in young versus old cells. In young cells, lncRNAs like ANRIL mediate association of polycomb repressive complexes with chromatin. In old cells, trithorax (MLL) derepressive complexes are associated instead, resulting in increased transcription. (Image adapted from O'Sullivan RJ, Karlseder J. The great unravelling: chromatin as a modulator of the aging process. Trends Biochem Sci. 2012; 37(11):466-76.)

Did you know?

Epigenetic alterations can occur via changes in DNA methylation, RNA interference, and Histone modifications.

New Publications

- Grammatikakis I, Panda AC, Abdelmohsen K, Gorospe M. Long noncoding RNAs(lncRNAs) and the molecular hallmarks of aging. Aging (Albany NY). 2014; 6(12):992-1009.
- Yuan T, Jiao Y, de Jong S, Ophoff RA, Beck S, Teschendorff AE. An Integrative Multi-scale Analysis of the Dynamic DNA Methylation Landscape in Aging. PLoS Genet. 2015; 18;11(2):e1004996.
- Bierhoff H, Dammert MA, Brocks D, Dambacher S, Schotta G and Grummt I. Quiescence-induced LncRNAs trigger H4K20 trimethylation and transcriptional silencing. Mol Cell. 2014; 54:675-682.

We are a lot like our cells...

Just like our cells undergo unravelling of heterochromatin, as we age, our short-term memory starts to unravel. We make longer to-do lists; cells make longer "to-transcribe" lists.

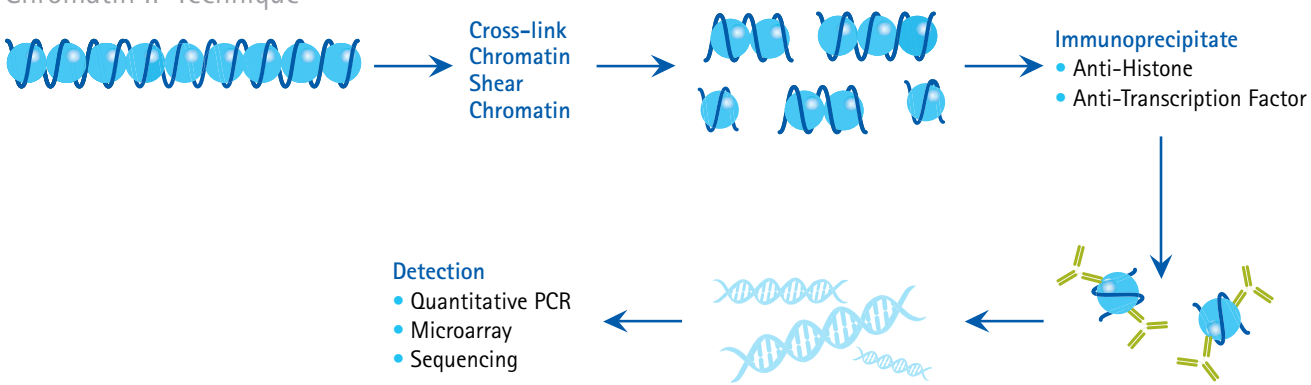
Epigenetic Alteration Detection

Featured Technique:

Chromatin Immunoprecipitation (ChIP)

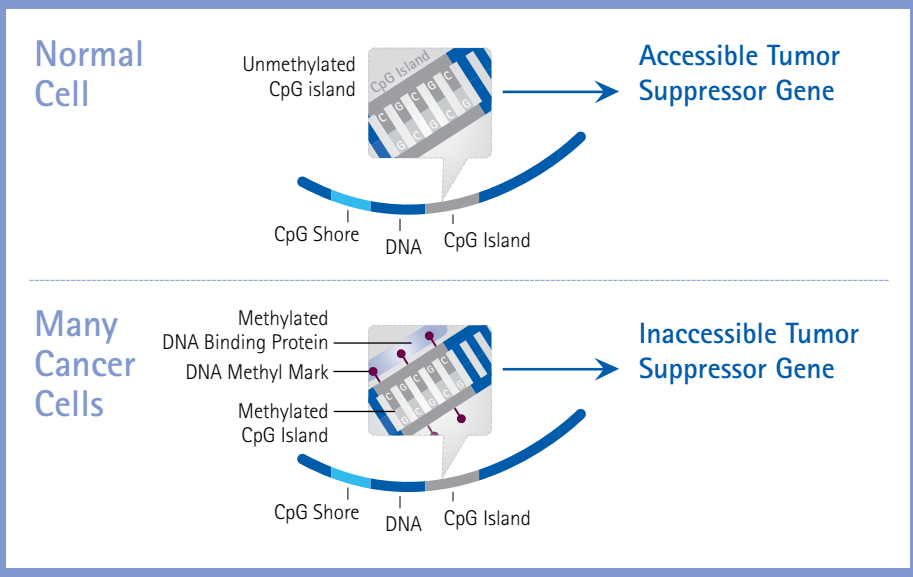
Chromatin Immunoprecipitation (ChIP) is a powerful technique for mapping the *in vivo* distribution of proteins associated with chromosomal DNA. These proteins can be histone subunits and post-translational modifications or other chromatin associated proteins such as transcription factors, chromatin regulators, etc. Additionally, ChIP can be used to identify regions of the genome associated with these proteins, or conversely, to identify proteins associated with a particular region of the genome. ChIP methodology often involves protein-DNA and protein-protein cross-linking, fragmentation of the cross-linked chromatin, and subsequent immunoprecipitation of chromatin with an antibody specific to a target protein. The DNA fragments isolated in complex with the target protein can be identified by a variety of methods including PCR, DNA microarray and DNA sequencing.

Chromatin IP Technique



The Hallmark & Disease

As we age, the environmental influences that lead to epigenetic alterations build up. The level of methylation on histones, DNA, and associated proteins regulates gene expression. For instance, hypermethylation of CpG islands in the promoter regions of tumor suppressor genes renders them inaccessible to the transcriptional machinery, and prevents their expression. There is a strong correlation between the hypermethylation of CpG islands and cancer.



Featured Solution:

Magna ChIP® HiSens Chromatin Immunoprecipitation Kit

(Catalogue No. 17-10460)

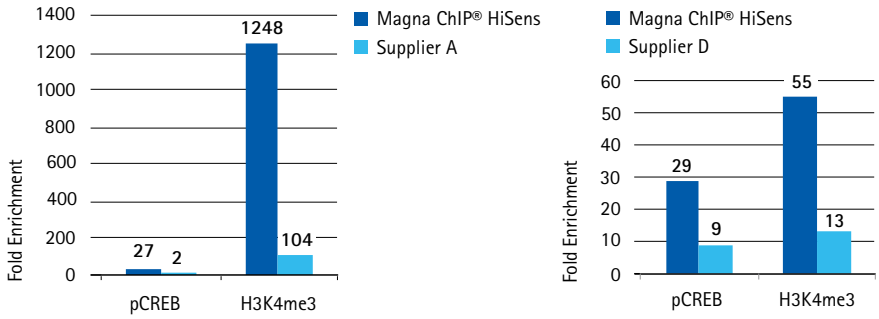
The Magna ChIP® HiSens kit provides a complete set of validated, quality controlled reagents, and a detailed protocol to enable ChIP from a wide range of input amounts of chromatin obtained from either cells or tissues. Our specialized blend of protein A/G blend of magnetic beads is specifically produced for chromatin immunoprecipiptation and enables the use of a broader range of antibodies than protein A or G alone eliminating the need to purchase different kits for different antibody isotypes. The SCW Buffer is unique to the Magna ChIP® HiSens kit and enables the use of a single buffer for multiple steps of the ChIP process (sonication, chromatin immunoprecipitation, and wash). The ChIP elution buffer provided with the HiSens kit has been formulated to allow analysis of enrichment by qPCR without additional clean-up steps for more rapid results. The materials provided allow twelve chromatin preparations and up to 24 ChIP assays.

Features and Advantages

- Robust ChIP from as few as 10,000 to as many as 1,000,000 cells
- Protocols and reagents for generation of chromatin from range of input sample type
- Superior performance with variety of isotypes of either polyclonal or monoclonal antibodies
- Specialized buffer and bead formulations; lower backgrounds and higher fold enrichment
- Single buffer system for sonication, chromatin IP, and wash
- Perform analysis of enrichment without additional purification after cross link reversal.
- Compatible with all commonly used downstream analysis applications – qPCR, next generation sequencing, microarray

Did you know?

Epigenetics is a mechanism by which our environment can influence our genome. Nature or Nurture are not independent. Our experience and environment modify our genes via epigenetic changes.
Unlike genetic mutations, epigenetic changes can be reversed, which gives us hope for curing diseases that are a result of such changes.
However, epigenetic changes can be inherited. For instance, smoking causes changes in miRNA expression in human sperm cells. Such epigenetically induced changes can be passed on to, and harm, the offspring.



Performance of the Magna ChIP® HiSens kit was benchmarked against that of other low input ChIP kits. After strictly following the protocols for all kits evaluated, the Magna ChIP® HiSens kit showed significantly higher performance as measured by fold enrichment and background levels than competitor kits

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Epigenetic Alteration:

Research	Description	Cat. No.
DNA Methylation	CpGenome™ Direct Prep Bisulfite Modification Kit (50 Reactions)	17-10451
	CpGenome™ Turbo Bisulfite Modification Kit	S7847
	CpGenome™ 5-hmC Quantitation Kit	17-10091
	CpG MethylQuest DNA Isolation Kit	17-10035
Chromatin Preparation	PureEpi™ Chromatin Preparation and Optimization Kit	17-10082
	EZ-Zyme™ Chromatin Prep Kit	17-375
Chromatin Immunoprecipitation (ChIP)	Chromatin Immunoprecipitation (ChIP) Assay Kit	17-295
	Magna ChIP® A - Chromatin Immunoprecipitation Kit	17-610
	Magna ChIP® G - Chromatin Immunoprecipitation Kit	17-611
	Magna ChIP® A/G Chromatin Immunoprecipitation Kit	17-10085
	Magna ChIP® G Tissue Kit	17-20000
	Magna ChIP® HT96 ChIP and Thermal Plate Set	17-10457
	Magna ChIP® Protein A Magnetic Beads	16-661
	Magna ChIP® Protein G Magnetic Beads	16-662
	Magna ChIP® Protein A+G Magnetic Beads	16-663
RNA Immunoprecipitation (RIP)	EZ-Magna ChIRP™ RNA Interactome Kit -Isolation and characterization of non-coding RNA:chromatin complexes	17-10495
	EZ-Magna Nuclear RIP™ (Cross-Linked) Nuclear RNA-Binding Protein Immunoprecipitation Kit	17-10521
	EZ-Magna Nuclear RIP™ (Native) Nuclear RNA-Binding Protein Immunoprecipitation Kit	17-10523
	EZ-Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit	17-701
Related Antibody & Controls	ChIPAb+™ Trimethyl-Histone H3 (Lys27) - ChIP Validated Antibody and Primer Set	17-622
	ChIPAb+™ Trimethyl-Histone H3 (Lys4) -ChIP Validated Antibody and Primer Set, rabbit monoclonal	17-614
	ChIPAb+™ RNA Pol II - ChIP Validated Antibody and Primer Set	17-620
	ChIPAb+™ REST - ChIP Validated Antibody and Primer Set	17-10456
	ChIPAb+™ Acetyl-Histone H3 (Lys9/18) - ChIP Validated Antibody and Primer Set	17-10241
	AccuChIP™ Trimethyl-Histone H3 Internal Control(Lys27) Chromatin Immunoprecipitation	17-10502
	AccuChIP™ Trimethyl-Histone H3 (Lys4) Chromatin Immunoprecipitation Internal Control	17-10505

Continued on next page

EMD Millipore offers effective solutions for research on topics related to Epigenetic Alteration (continued):

Research	Description	Cat. No.
Chip-Seq NGS Kits	PureGenome™ Low Input NGS Library Construction Kit	17-10492
	Magna ChIP-Seq™ ChIP and NGS Library Preparation Kit	17-1010
Histone Peptide Arrays	AbSurance® Histone H2A, H2B, H4 Antibody Specificity Array	16-665
	AbSurance® Histone H3 Antibody Specificity Array	16-667
	AbSurance® Complete Core Histone Antibody Specificity Array	16-668
	AbSurance® Pro Histone Peptide Microarray	16-671
Histone Proteins	Histone H1 Protein, 20 mg	14-155
	Histone H3, human recombinant	14-494
	Core Histones	13-107
	Core Histones, HeLa	13-183
Related Small Molecules	SRT1720	567860
	HMTase Inhibitor V, UNC0224	382193
	HMTase Inhibitor II, Chaetocin	382191
	JMJD2 Inhibitor, 5-carboxy-8HQ	420201
	JMJD Histone Demethylase Inhibitor III	420202
	Histone Methyltransferase EZH2 Inhibitor, DZNep	252790
	Histone Deacetylase Inhibitor IV	382170
HDAC Assays	SIRTainty® Class III HDAC Assay	17-10090
	HDAC Assay Kit, colorimetric detection	17-374
	HDAC Assay Kit, fluorometric detection	17-356
	Histone Deacetylase Assay Kit (HDAC)	17-320

For a complete selection, visit: www.emdmillipore.com/epigenetics

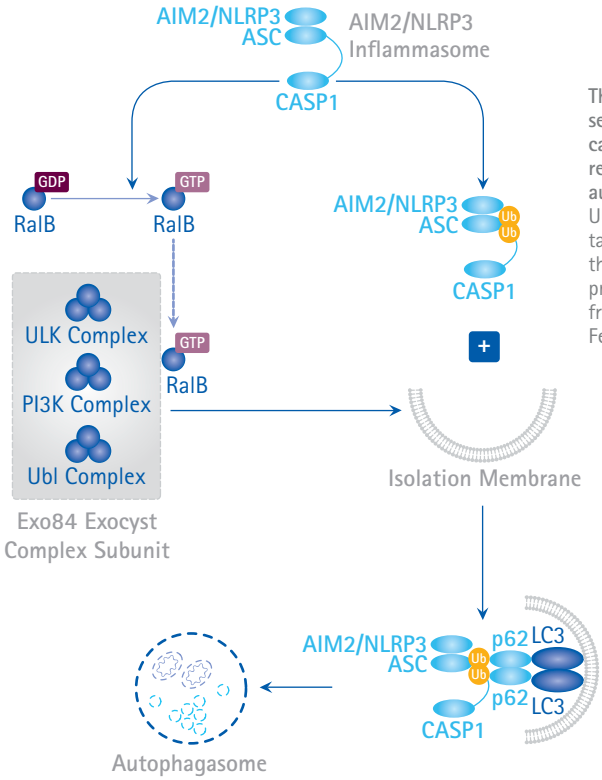
Notes

Loss of Proteostasis

As cells age, environmental stresses add up and mechanisms responsible for maintaining proper protein composition start to decline. Proteins lose their stability, autophagic processes start to fail, and misfolded proteins accumulate.

Over the years, our bodies are subjected to many environmental inputs that put thermal stress, oxidative stress, and osmotic stress on our cells, causing misfolding of proteins. For example, free radicals present in polluted air have been identified as particularly noxious agents in this regard, contributing to multiple aging-related pathologies. In younger cells, micro- and macroautophagy pathways, together with the ubiquitin-proteasome system, take care of clearing these unfolded proteins. However, in aging cells, autophagy induction can be gradually compromised, and lysosomes become less efficient at eliminating the vesicles carrying this cellular waste.

In a vicious cycle termed "inflammaging," this decrease in efficient autophagy results in an increase in intracellular ROS, triggering the damage-sensing inflammasome to generate low levels of chronic inflammation, further accelerating aging.



The inflammasome's damage-sensing proteins, AIM2 or NLRP3, cause RalB to bind to Exo84, resulting in nucleation of the autophagosome membrane. Ubiquitinated inflammasomes are tagged for autophagic destruction, thereby limiting the secretion of proinflammatory cytokines. (adapted from Oh JE. Immune Netw. 2013 Feb;13(1):1-9.)

Did you know?

There are three forms of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy.

The failure of autophagy is thought to be one of the main reasons for the accumulation of cell damage and aging.

New Publications

- Liu K, Zhao E, Ilyas G, Lalazar G, Lin Y, Haseeb M, Tanaka KE, Czaja MJ. Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. Autophagy. 2015; 11(2):271-84.
- Tramutola A, Triplett JC, Di Domenico F, Niedowicz DM, Murphy MP, Coccia R, Perluigi M, Butterfield DA. Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. J Neurochem. 2015;133(5):739-49.
- Numan MS, Brown JP, Michou L. Impact of air pollutants on oxidative stress in common autophagy-mediated aging diseases. Int J Environ Res Public Health. 2015;12(2):2289-305.

We are a lot like our cells...

Just like our cells accumulate misfolded proteins, as we age, we accumulate mementoes of our past, filling our closets. We try to de-clutter and stay organized, our cells' autophagic pathways attempt to keep up with the cellular clutter.

Autophagy Detection

Featured Technique:

Lentiviral Biosensors

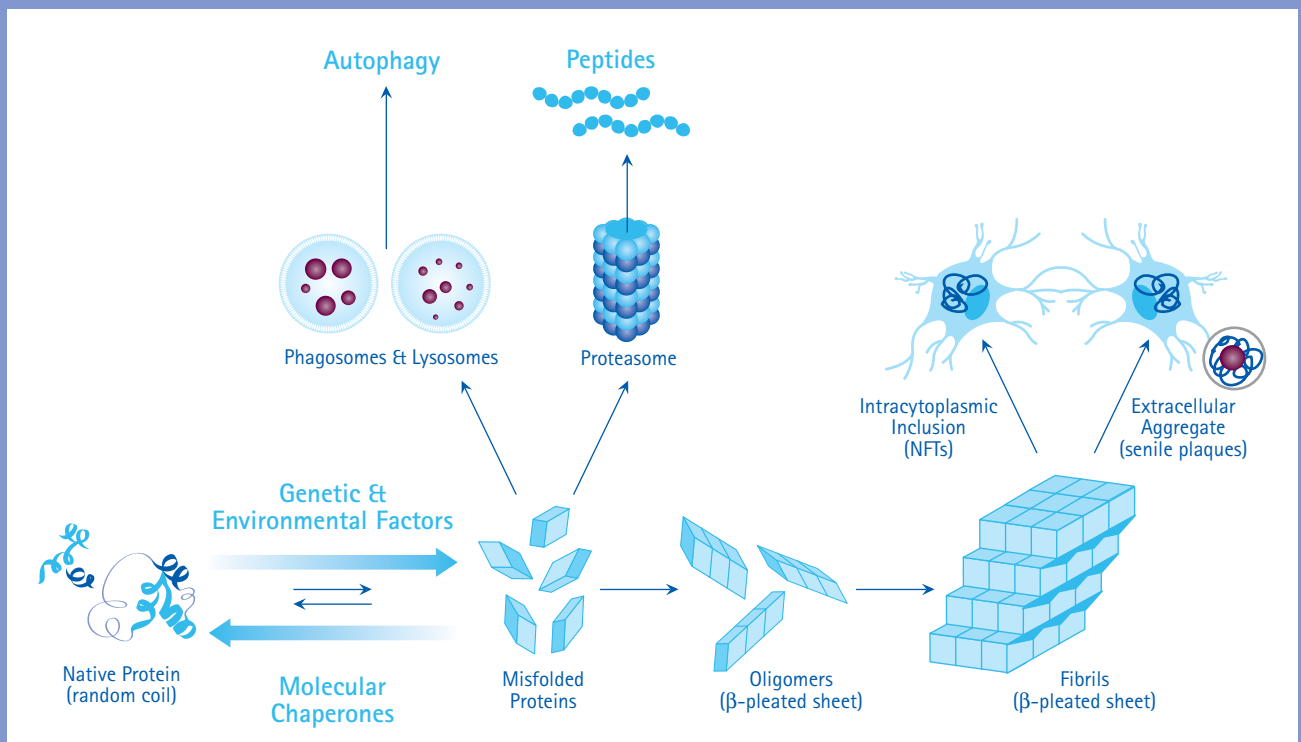
Biosensors can be used to detect a particular protein as well as the subcellular location of that protein within live cells. Fluorescent tags are an effective way to visualize the protein of interest within a cell by either fluorescent microscopy or time-lapse video capture. Visualizing live cells without disruption can reveal changing cellular conditions in real time.

Lentiviral vector systems are a popular tool for introducing genes and gene products into cells. Advantages over non-viral methods (such as chemical-based transfection) include higher-efficiency transfection of dividing and non-dividing cells, stable expression of the transgene, and low immunogenicity.

The Hallmark & Disease

As we age, the synthesis, folding, and degradation of proteins is perturbed. As a result, the production and accumulation of misfolded proteins that form aggregates increases, eventually resulting in disease.

Neurological diseases related to the amyloid protein are very common, and include Alzheimer's disease, Parkinson's disease, and Huntington's disease. Another protein, the prion protein is responsible for transmissible spongiform encephalopathies, or TSEs. These include infectious diseases such as scrapie in sheep, bovine spongiform encephalopathy (mad cow disease, the infective form of which can cause Creutzfeldt-Jakob disease in humans) and kuru, the only epidemic human prion disease known.



Featured Solution:

LentiBrite™ Lentiviral Biosensors for LC3 and p62 with GFP and RFP

EMD Millipore's LentiBrite™ Lentiviral Biosensors, a new suite of pre-packaged lentiviral particles encoding foundational proteins of autophagy detection - LC3 and p62, enabling precise visualization of autophagosome formation under different cell/disease states in live cell and *in vitro* analysis. Visualize autophagy in real time, even in difficult-to-transfect cell types, using LentiBrite™ GFP- & RFP-tagged LC3 and p62 wild-types and LC3-G120A mutant control lentiviral biosensors.

Advantages:

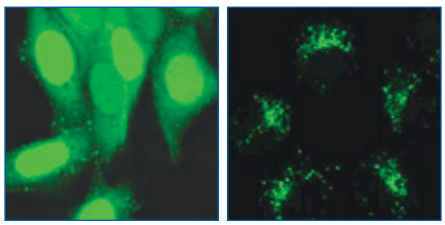
- Pre-packaged, ready-to-use, fluorescently-tagged LC3 and p62 with monomeric GFP and RFP
- Minimum titer (≥3 x 10^8 IFU/mL) per vial
- Long-term, stable fluorescent expression that is non-disruptive towards cellular function
- Higher efficiency transfection as compared to traditional chemical-based and other non-viral-based transfection methods
- Ability to transfect dividing, non-dividing, and difficult-to-transfect cell types, such as primary cells or stem cells
- Validated for fluorescent microscopy and live cell analysis
- LC3 Control Mutant lentiviral particle contains the translocation-defective protein LC3-G120A for comparison studies.

Did you know?

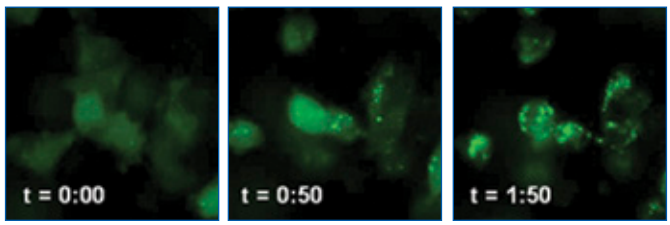
Proteostasis is maintained, in part, through the action of small heat-shock proteins (sHsps). These intra-cellular molecular chaperone proteins play an important role in preventing protein aggregation, as may occur when cells experience elevated temperature or oxidative stress.

Description	Cat. No.
LentiBrite™ GFP-LC3 Lentiviral Biosensor	17-10193
LentiBrite™ RFP-LC3 Lentiviral Biosensor	17-10143
LentiBrite™ GFP-LC3 Control Mutant Lentiviral Biosensor	17-10189
LentiBrite™ RFP-LC3 Control Mutant Lentiviral Biosensor	17-10188
LentiBrite™ GFP-p62 Lentiviral Biosensor	17-10224
LentiBrite™ RFP-p62 Lentiviral Biosensor	17-10404

Uninduced Induced



Using lentiviral biosensors to detect autophagy. HeLa cells were treated with lentivirus and left in complete media (Uninduced) or incubated in EBSS with lysosome inhibitor (Induced). Images were obtained by oil immersion confocal fluorescence microscopy.



Time-lapse Imaging of GFP-LC3 in autophagosomes in autophagic cells. HT-1080 cells were treated as described for figure above. Shortly following initiation of starvation conditions, time-lapse imaging was performed under temperature-controlled oil immersion wide-field fluorescence microscopy, with images taken every 1 minute over the course of 2 hours. Images demonstrate the translocation of GFP-LC3 from diffuse nuclear/cytosolic localization to discrete cytoplasmic puncta.

Watch a video of autophagy occurring in real time at: www.emdmillipore.com/lentibritebiosensor

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Proteostasis:

Research	Description	Cat. No.
Autophagy	LC3-II Enrichment Kit (Western Blot)	17-10232
	LentiBrite™ GFP-LC3-II Enrichment Kit (Flow Cytometry)	17-10230
	FlowCellect® GFP-LC3 Reporter Autophagy Assay Kit (CHO)	FCCH100170
	FlowCellect® Autophagy LC3 Antibody-based Assay Kit (100 tests)	FCCH100171
	FlowCellect® GFP-LC3 Reporter Autophagy Assay Kit (U2OS)	FCCH100181
	FlowCellect® RFP-LC3 Reporter Autophagy Assay Kit	FCCH100183
	FlowCellect® Autophagy Detection Reagent Pack, 100 tests	CF200097
Proteosome Kits & Assays	Protease Assay Kit	539125
	Proteasome isolation Kit	5391776
	20S Proteasome Assay Kit	APT280
	Ubiquinated Protein Enrichment Kit	662200
Related Antibodies	Anti-LC3A Antibody, clone EPR1754 (MABC175)	MABC175
	Anti-LC3A/B (N-term) Antibody, clone EP1983Y, Rabbit Monoclonal	MABC176
	Anti-LC3A (N-term) Antibody, clone EP1528Y, Rabbit Monoclonal	MABC177
	Anti-ATG3 Antibody	AB2953
	Anti-ATG4C Antibody	ABC21
	Anti-ATG5 Antibody	ABC14
	Anti-ATG5 Antibody	AB15404
	Anti-ATG5 Antibody	AB15404P
	Anti-ATG5 Antibody, clone 177.19	MAB2605
	Anti-ATG7 Antibody	AB10511
	Anti-ATG7 Antibody, clone EP1759Y	04-1055
	Anti-ATG9 L2 Antibody	AB15407
	Anti-UVRAG Antibody	AB2960
	Anti-Beclin-1 (CT) Antibody, clone EPR1733Y, Rabbit Monoclonal	MABN16
Related Small Molecules	AG 9	658390
	AG 112	658440
	Akt Inhibitor IV	124011
	Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2	124018
	Akt Inhibitor X	124020
	Bisindolylmaleimide I	203290
	PI-103	528100
	PI 3-Kγ Inhibitor	528106
	PKCß Inhibitor	539654
	Rapamycin	553210
	Rho-Kinase Inhibitor III, Rockout	555553
	SU11652	572660

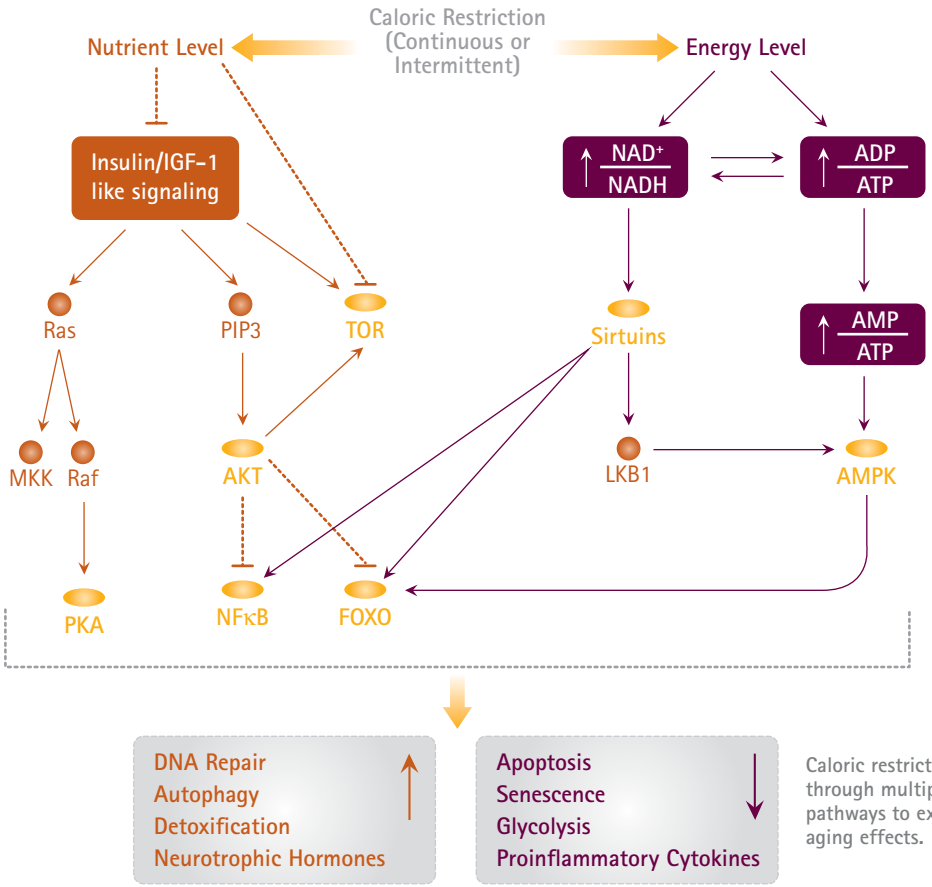
For a complete selection, visit: www.emdmillipore.com/autophagy

Deregulated Nutrient Sensing

Metabolic activities can put stress on our cells. Too much activity, and changes in nutrient availability and composition cause cells to age faster.

Metabolism and its byproducts, over time, damage cells via oxidative stress, ER stress, calcium signaling, and mitochondrial dysfunction. Therefore, organisms depend on multiple nutrient sensing pathways to make sure that the body takes in just the right amount of nutrition – not too much, not too little. However, these damaging events also deregulate the nutrient-sensing molecules and downstream pathways. A misguided hypothalamus may signal for greater food intake, then, when the body doesn't really require it. Age-related obesity, diabetes and other metabolic syndromes result. To make things even worse, obesity- and diabetes-related chronic inflammation, operating via JNK and IKK crosstalk, can deregulate nutrient sensing further.

Probably because so many interdependent pathways link metabolism to aging, these are the pathways that have received the most intense focus in the search for anti-aging therapeutics. There was much excitement in the last decade around resveratrol and caloric restriction, the effects of which have now been shown to be limited to mice and other model organisms. Today, intermittent caloric restriction (i.e., fasting) is the only intervention that has been shown to extend human lifespan.



Caloric restriction operates through multiple signaling pathways to exert anti-aging effects.

Did you know?

Blueberries are a wonderful source of healthy nutrients, and is one of the world's healthiest foods. They provide a long list of bioactive compounds that serve as antioxidants, vitamins, and minerals. Blueberries are known to help with maintaining healthy bones, lowering blood pressure, managing diabetes, preventing cancer, improving mental health, and healthy digestion.

New Publications

- de Cabo R, Carmona-Gutierrez D, Bernier M, Hall MN, Madeo F. The search for antiaging interventions: from elixirs to fasting regimens. Cell. 2014; 157(7):1515-26.
- Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. Cell Metab. 2014 ;19(2):181-92.
- Cheng CW, Adams GB, Perin L, Wei M, Zhou X, Lam BS, Da Sacco S, Mirisola M, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell. 2014;14(6):810-23.

We are a lot like our cells...

Just like our cells suffer from difficulties with nutrient sensing, as we age, eating too much sugar stresses our brain. Keep your mental acuity by eating less a couple days a week – it's the only proven way to live longer.

The Hallmark & Disease

Inflammation is regulated, in part, by Sirt1 by way of its inhibition of NFκB, a promoter of the inflammatory response. Inflammation is involved in a number of diseases such as cancer, arthritis, asthma, heart disease, and neurodegenerative events.

Did you know?

Exercise and caloric restriction leads to increased longevity by way of reduced replication stress, reduced DNA damage, reduced inflammatory signals, and overall reduced metabolic activity and damage inducing by-product production.

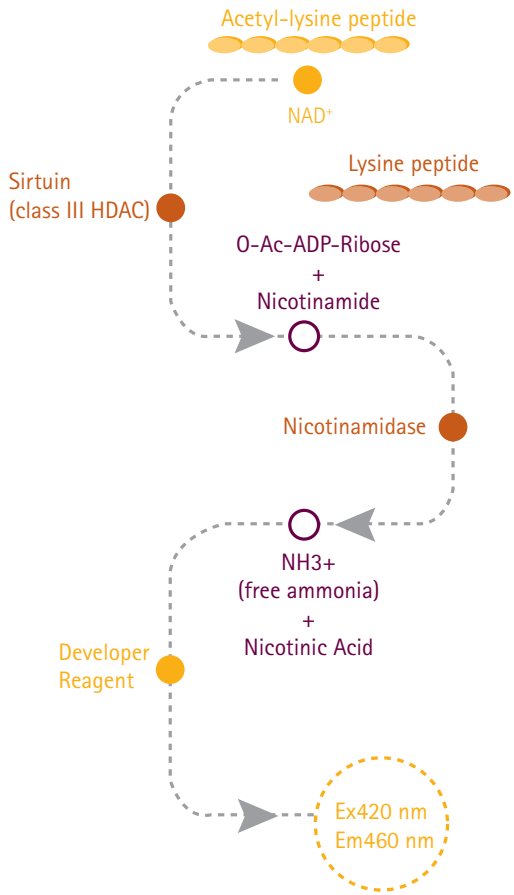
Sirtuin Detection

Featured Technique:

Sirtuin HDAC Assays

Class III histone deacetylases (HDACs), also known as sirtuins, are mechanistically distinct from class I and class II HDACs in that they couple deacetylation of the peptide/protein substrate to cleavage of NAD+ to form nicotinamide and O-acetyl-ADP-ribose. The mammalian sirtuins consist of 7 members, termed Sirt1-7, which share the NAD+-binding catalytic domain, but differ in N- and C-termini, subcellular localization, substrate preference, and biological function. Sirtuins have been intensely researched since it was discovered that their activation led to reduced incidence of aging and age-related diseases such as diabetes.

To better understand the biological roles of sirtuins in nutrient sensing, researchers would benefit from an alternative assay that uses untagged, native peptide substrates, enabling the study of sirtuins without the complication of fluorophore-mediated activation. To measure sirtuin activity in a more physiologically relevant manner and address the diversity of sirtuin isoforms and potential substrates, the SIRTainty® assay platform enables the analysis of sirtuin activity using virtually any appropriate substrate.



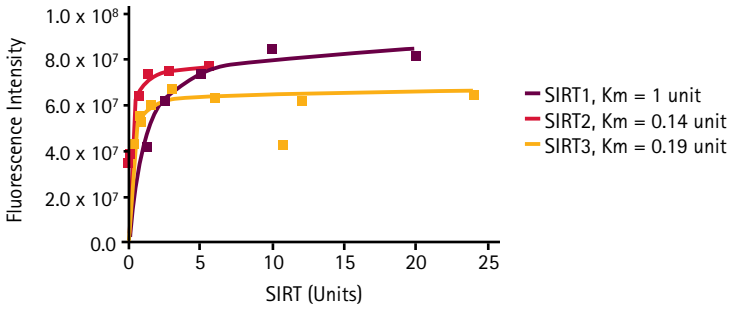
SIRTainty® assay principle. Sirtuin-mediated deacetylation of unlabeled peptide substrate generates nicotinamide as a product. The SIRTainty® assay couples sirtuin enzyme activity to nicotinamidase, which cleaves nicotinamide into nicotinic acid and free ammonia. A developer reagent is added, which reacts with the free ammonia to generate a fluorophore. The resulting fluorescent signal is quantified with a conventional fluorometric plate reader.

Featured Solution:

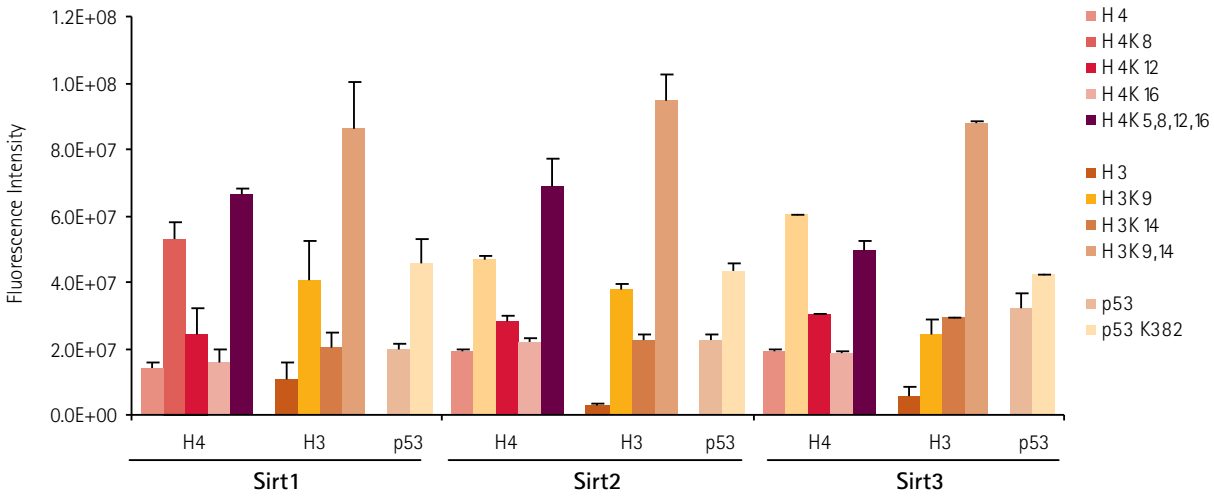
SIRTainty® Class III HDAC Assay

(Catalogue No. 17-10090)

This new SIRTainty® class III HDAC assay is a flexible, reliable, homogeneous, no-wash assay for quantifying sirtuin activity. Based upon novel, patent-pending technology, this easy-to-perform assay is coupled to nicotinamidase, which catalyzes breakdown of nicotinamide generated upon cleavage of NAD+ during sirtuin-mediated deacetylation of a substrate. Thus, the SIRTainty® assay provides a direct assessment of the activity of class III HDAC enzymes.



Sirtuin isoform activity. The SIRTainty® assay was effective in measuring activity of all three sirtuin isoforms tested. SIRT2 displayed the highest affinity (Km = 0.14 units) for the acetylated H3K9 substrate used.



Siruin substrate preference. Sirt1, Sirt2, and Sirt3 exhibit preference for acetylated (H3K9, H3K9/14, H4K8, and H4K5/8/12/16) versus nonacetylated peptides. Sirt1 and 2, but not Sirt3, demonstrated higher deacetylation activity with a human p53 peptide acetylated at K382 compared to the non-acetylated peptide.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Cellular Metabolism:

Research	Description	Cat. No.
Metabolism	MILLIPLEX® MAP Human Metabolic Hormone Magnetic Bead Panel	HMHEMAG-34K
Akt and mTOR Signaling	MILLIPLEX® MAP Human Akt/mTOR 11-Plex, Phosphoprotein	48-611MAG
	MILLIPLEX® MAP Human Akt/mTOR 11-Plex, Total Protein	48-612MAG
	MILLIPLEX® MAP Total mTOR Magnetic Bead MAPmate	46-685MAG
	MILLIPLEX® MAP Phospho mTOR (Ser2448) Magnetic Bead MAPmate	46-686MAG
	FlowCellect® PI3K-mTOR Signaling Cascade kit	FCCS025210
	K-LISA mTOR activity Kit	CBA055
	K-LISA mTOR Recombinant Activity Assay	CBA104
Sirtuins – Proteins	SIRT1, GST-Fusion, Human, Recombinant, *E. coli*	524743
	SIRT2, His•Tag®, Human, Recombinant, *E. coli*	524744
	SIRT3, GST-Fusion, Human, Recombinant, *E. coli*	524745
	SIRT5 (His-tag) human recombinant - HDAC protein	03-230
Sirtuins – Antibodies	Anti-SIRT1 Antibody, clone 10E4	04-1557
	Anti-Sir2/SIRT1 (NT) Antibody, clone E54, rabbit monoclonal	04-1013
	Anti-Sir2/SIRT1 Antibody, clone E104, rabbit monoclonal	04-1091
	Anti-SIRT2 (CT) Antibody, clone EP1668Y, rabbit monoclonal	04-1124
	Anti-SIRT2 Antibody	09-843
	Anti-SIRT3 (CT) Antibody	07-1596
	Anti-SIRT4 Antibody	ABE800
	Anti-SIRT4 Antibody, clone 4F9.1	MABE223
	Anti-Sirt5 Antibody	ABE198
	Anti-Sirt6 Antibody, clone 6G2.2	MABE142
Sirtuins – Small Molecules	Sirtinol	566320
	InSolution™ Sirtinol	566321
	SIRT1/2/3 pan Inhibitor	505479
	SIRT1 Inhibitor III	566322
	SIRT1/2 Inhibitor IV, Cambinol	566323
	SIRT2 Inhibitor, AGK2	566324
	SIRT1 Inhibitor IV, (S)-35	566325
	SIRT2 Inhibitor, Inactive Control, AGK7	566326
	SIRT1/2 Inhibitor VII	566327
	SIRT1/2 Inhibitor VIII, Salermide	566330
	SIRT2 Inhibitor II, AK-1	566331
	Sirt1 Inhibitor VII, Inauhzin	566332
Related Antibodies	Akt/mTOR/S6K Pathway Explorer Antibody MiniPack	15-104
	mTOR phosphorylation Pathway Explorer Antibody MiniPack	15-105

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

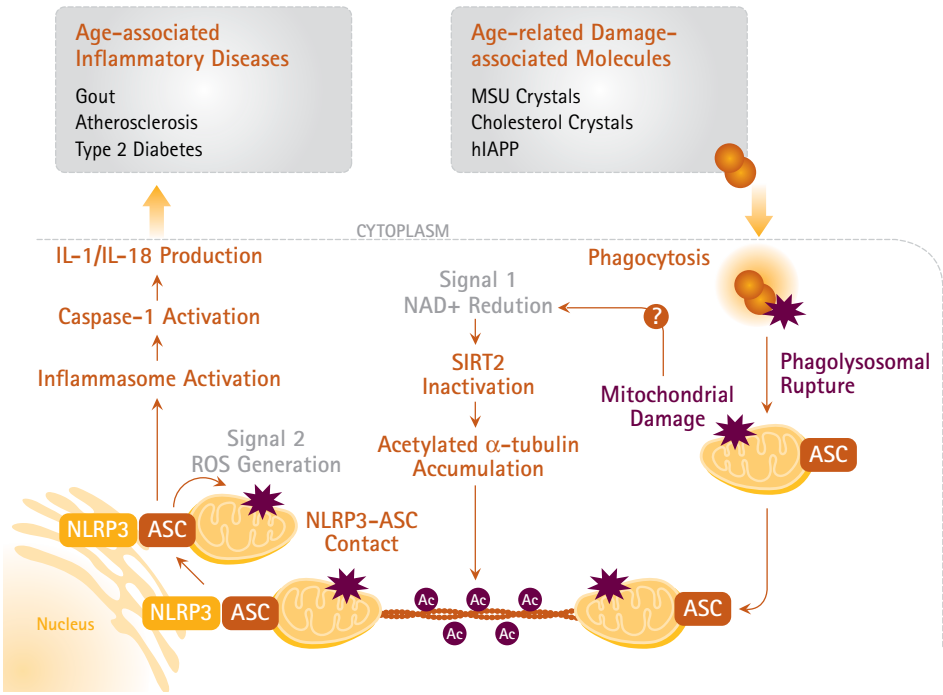
Mitochondrial Dysfunction

As cells age, their mitochondria start to lose their integrity due to the build-up of oxidative stress. Compromised mitochondrial function leads to a number of events, such as increased apoptosis induction, that correlate with aging.

Not only are mitochondria responsible for generating ATP, but they act as sensors of cellular distress, and are the first parts of the cell to send and respond to cell death signals. Since the 2013 publication of The Hallmarks of Aging, the role of mitochondria in regulating inflammation in response to metabolic change (via mitochondrial sirtuins) has received greater attention.

Mitochondria send signals via calcium signaling and reactive oxygen species (ROS) to the NF-κB pathway, as well as via damage-associated molecular patterns (DAMPs) to the inflammasome, to activate "inflammaging." In turn, proinflammatory molecules regulate mitochondria by causing decreases in mitochondrial membrane potential, a sign of poor cell health.

Melatonin signaling, on the other hand, affects the mitochondria positively, maintaining the integrity and function of these organelles. Melatonin decreases inflammation and maintains the efficiency of electron transport. Because of these powerful effects, maintaining melatonin levels via pharmaceuticals or by circadian regulation is an area of therapeutic interest. The future of mitochondrial dysfunction research will certainly include more genetic and epigenetic data around mtDNA, as well as their implications for innate immunity.



Did you know?

Approximately 3000 genes are needed to make a mitochondrion. Of these, mitochondrial DNA (mtDNA) encodes 37 genes, and the remaining are from nuclear DNA (nDNA).

Mitochondrial dysfunction caused by aging-related insults can direct chronic inflammation mediated by the inflammasome, which is a multiprotein complex that includes NLRP3 and ASC.

Adapted from Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, Akira S. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. Nat Immunol. 2013; 14(5):454-60.

New Publications

- Kapetanovic R, Bokil NJ, Sweet MJ. Innate immune perturbations, accumulating DAMPs and inflammasome dysregulation: A ticking time bomb in ageing. Ageing Res Rev. 2015 Feb 25. pii: S1568-1637(15)00028-8.
- Hardeland R, Cardinali DP, Brown GM, Pandi-Perumal SR. Melatonin and brain inflammaging. Prog Neurobiol. 2015; 127-128C:46-63.
- Liu TF, Vachharajani V, Millet P, Bharadwaj MS, Molina AJ, McCall CE. Sequential actions of SIRT1-RELB-SIRT3 coordinate nuclear-mitochondrial communication during immunometabolic adaptation to acute inflammation and sepsis. J Biol Chem. 2015; 290(1):396-408.

We are a lot like our cells...

Just like our cells find ways to reduce mitochondrial stress, as we age, we find ourselves finding ways to reduce stress and feel more at peace. Melatonin can help aging mitochondria cope with stress—and melatonin helps our entire bodies deal with life's stressors as well. A little bit of sunshine goes a long way.

Oxidative Stress Detection

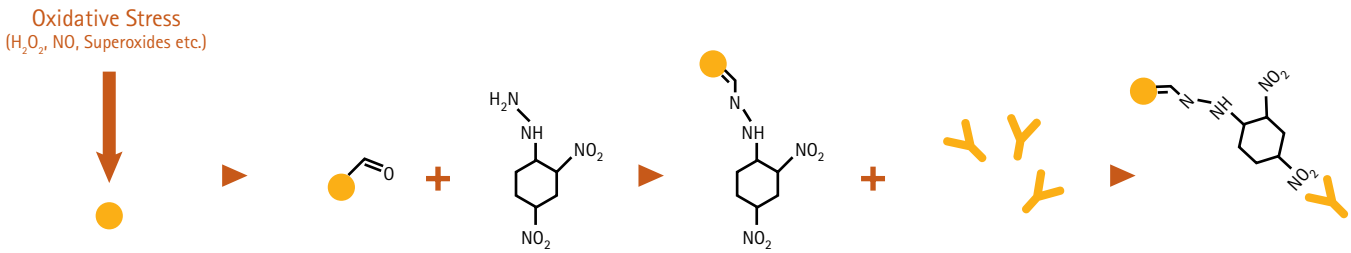
Featured Technique:

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 and have been implicated as one of the contributing factors of aging. In the case of proteins the ROS primarily cause carbonyl derivatives on amino acid sidechains affecting their enzymatic and biochemical functionality. Not surprisingly, carbonyl formation has become an important biomarker for oxidative stress.

Oxidative modification of proteins by oxygen free radicals and other reactive species such as hydroxynonenal occurs in physiologic and pathologic processes. As a consequence of the modification, carbonyl groups are introduced into protein side chains by a site-specific mechanism. EMD 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.



Did you know?

While mitochondria are responsible for creating over 90% of the energy needed for survival and growth, only about 3% of the genes necessary to make a mitochondrion are allocated for making ATP.

Featured Solutions:

OxyBlot™ Protein Oxidation Detection Kit

(Catalogue No. S7150)

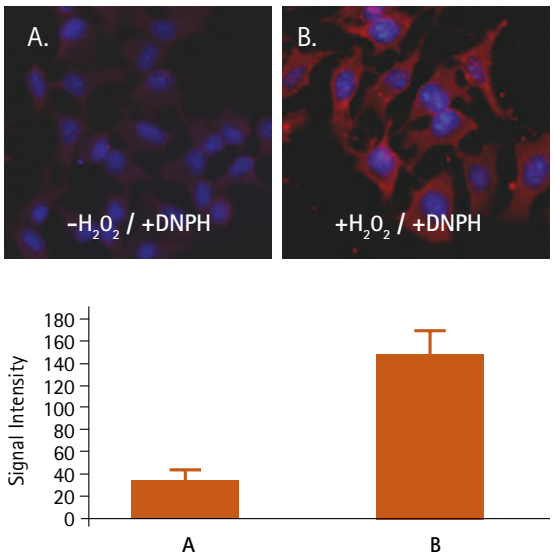
The OxyBlot™ Protein Oxidation Detection Kit provides the chemical and immunological reagents necessary to perform the immunoblot detection of carbonyl groups introduced into proteins by oxidative reactions with ozone or oxides of nitrogen or by metal catalyzed oxidation.

Under the conditions recommended in the kit, as little as 5 femtomoles of carbonyl residue can be detected. This sensitivity is at least 100 times greater than that obtained by other procedures (such as radioisotope methodology. In addition, you can quantitatively analyze the oxidative status of each protein by comparing the signal intensity of the same protein in different lanes on the same or different gels.

OxyICC™ Oxidized Protein Detection Kit

(Catalogue 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.

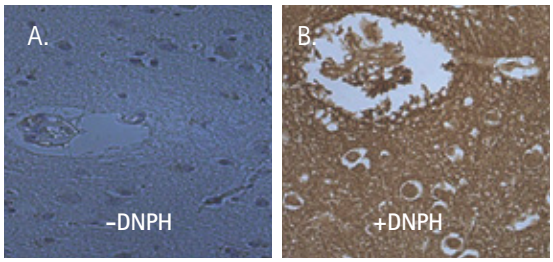


Quantitation of OxyICC™ Analysis. Experiments were performed using HeLa cells and hydrogen peroxide (H_2O_2) treatment (400 µM for 30 minutes). The cells were then analyzed for oxidative stress following the OxyICC™ protocol. For each test reaction the Cy3 fluorescence pixel intensity was quantified 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 was 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).

The OxyIHC™ Oxidative Stress Detection Kit

(Catalogue No. S7450)

The OxyIHC™ Oxidative Stress Detection Kit contains the chemical and immunological reagents necessary to detect protein oxidation in various tissues from a variety of organs and animal species. The DNP-derivatized proteins are detected using an antibody that specifically binds to the DNP moiety. Subsequent incubation with biotin conjugated secondary antibody, streptavidin conjugated HRP, and development using a 3,3' diaminobenzidine (DAB) staining allows immunohistochemical detection of protein oxidation.



OxyIHC™ identified oxidative stress in the brain of an Alzheimer patient. The brain tissue was fixed and stained according to the OxyIHC™ protocol. Negative control reactions were performed with the Derivatization Control Solution and showed minimal DAB reactivity with only Hematoxylin staining (panel A). Staining with DNPH resulted in strong immunoreactivity (panel B).

The Hallmark & Disease

Parkinson disease (PD) is associated with progressive loss of dopaminergic neurons in the substantia nigra, as well as with more-widespread neuronal changes that cause complex and variable motor and non-motor symptoms. Impaired mitochondrial function is likely to increase oxidative stress and might render cells more vulnerable to this and other related processes, including excitotoxicity. A complex interplay occurs between mitochondria and other cellular machinery that affects cell survival, as mitochondria not only have a key role in electron transport and oxidative phosphorylation, but they are also the main cellular source of free radicals, and they are involved in calcium homeostasis and in the regulation and instigation of cell-death pathways.

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Mitochondrial Dysfunction:

Research	Description	Cat. No.
Oxidative Stress	Superoxide Dismutase Assays	574601
	OxyBlot™ Protein Oxidation Detection Kit	S7150
	OxyICC™ Oxidized Protein Detection Kit	S7350
	OxyIHC™ Oxidative Stress Detection Kit	S7450
	Glutathione Detection Kit	APT250
	FlowCellect® MitoStress Kit	FCCH100109
	FlowCellect® Oxidative Stress Characterization Kit	FCCH025111
	MILLIPLEX® MAP Human Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	HOXPSMAG-16K
	MILLIPLEX® MAP Rat/Mouse Oxidative Phosphorylation (OXPHOS) Magnetic Bead Panel	RMOXPSMAG-17K
	NovaQUANT® Human Oxidative Stress qPCR Kit	72627
	NovaQUANT® Mouse Oxidative Stress qPCR Kit	72628
	Superoxide Dismutase Assays	574061
Mitochondrial Health	MitoLight® Mitochondrial Apoptosis Detection Kit (4500-0250)	APT142, APT242
	Guava® Mitochondrial Depolarization Kit for Flow Cytometry	4500-0250
	FlowCellect® MitoPotential Red Kit	FCCH100105
	FlowCellect® MitoLive Kit (7)	FCCH10010
	FlowCellect® MitoDamage Kit	FCCH100106
	FlowCellect® Cytochrome C Kit	FCCH100110
	Mitochondrial Complex Activity Assay Kits	AAMT001 - AAMT006
	Muse® Mitopotential Assay Kit	MCH100110
Mitochondria Isolation	Mitochondria/Cytosol Fractionation Kits	MIT1000, QIA88
Nuclear DNA Ratio	NovaQUANT® Human Mitochondrial to Nuclear DNA Ratio Assay	72620
	NovaQUANT® Mouse Mitochondrial to Nuclear DNA Ratio Assay	72621
Related Small Molecules	Mitochondrial Citrate Transport Protein (CTP) Inhibitor	475877
	Mitochondrial ATF Translocator, SBI-0087702	505988
	Rotenone	557368
	Bax Channel Blocker II, iMAC2	196806
	Daunorubicin, Hydrochloride	251800

Continued on next page

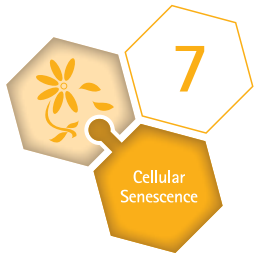
EMD Millipore offers effective solutions for your research on areas related to Mitochondrial Dysfunction (continued):

Research	Description	Cat. No.
Related Antibodies	WideScreen® Mitchondrial Total OXPhos Human WB Antibody Cocktail	ACMT001
	WideScreen® Mitchondrial OXPhos Rodent WB Antibody Cocktail	ACMT002
	Anti-Mitochondria Antibody	AB3598
	Anti-Mitochondria Antibody, clone 113-1, Alexa Fluor® 488 conjugate	MAB1273A4
	Anti-Mitochondria Antibody, clone 113-1, Biotin Conjugate	MAB1273B
	Anti-Mitochondria Antibody, surface of intact mitochondria, clone 113-1	MAB1273
	Anti-ETFA Antibody	ABS6080
	Anti-ATP Synthase Antibody, β chain, clone 4.3E8.D1	MAB3494
	Anti-CYP24A1 Antibody	ABN201
	Anti-CYP27A1 Antibody	ABC420
	Anti-Cytochrome P450 (scc) Antibody, a.a. 509-526	ABS236
	Anti-PITRM1 Antibody	ABT303
	Anti-Peroxiredoxin-5 Antibody (PRDX5), clone 5 288 2F4	MABN301
	Anti-PRDX5 Antibody	ABC281
	Anti-Cytochrome C Antibody, clone EP1326Y, rabbit monoclonal	04-1043
	Anti-AIF (Apoptosis Inducing Factor) Antibody	07-208
	Anti-Smac/DIABLO Antibody, clone Y12, rabbit monoclonal	04-578
Reagents, Stains, & Solutions	MTT Reagent A	CT01-5
	MTT	475989
	JC-1	420200
	Chenodeoxycholic Acid, Sodium Salt	220411
	Nigericin, Sodium Salt, Streptomyces hygroscopicus	481990
	Creatine Phosphate, Disodium Salt	2380

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

Notes

Cellular Senescence

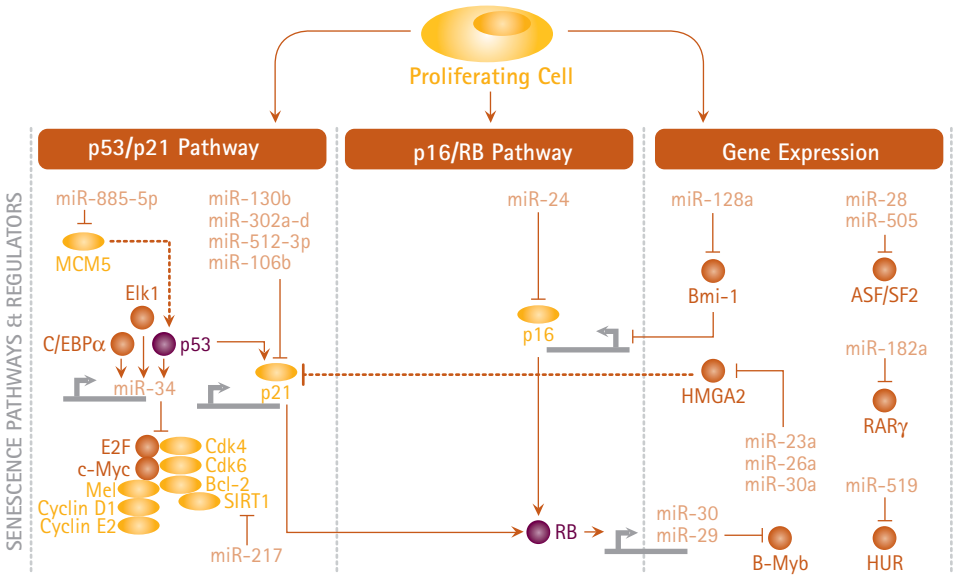


Cellular senescence is the point at which our cells stop dividing and growing due to damage or lack of necessary components. As cells age, they lose their ability to actively divide and start to undergo senescence.

Senescence refers to a pause in the cell cycle, usually in response to damage. Young senescent cells are presumably cleared by the immune system, but in older tissues, they stick around, secreting harmful proinflammatory signals like IL-6 and IL-8 that damage our bodies further.

As one would guess, the DNA damage and oxidative stress responses, via p53 and AMPK, induce senescence via the retinoblastoma pathway. And telomere shortening causes cells to stop replicating, reinforcing the senescent phenotype. The cell cycle regulator, p16, plays a still-mysterious role in aging-related senescence. Levels of p16 are more correlated to chronological age than are the levels of any other protein, but its precise regulation in the context of aging is not clear. Developmental signaling, such as by the Id proteins and microRNAs, is involved.

The emerging theme is that it's not enough to measure one or two biomarkers to unequivocally define the senescent state. To fully characterize aging cells, measure at least a few of the following phenotypic markers: secretory phenotype, beta galactosidase expression, proliferation, heterochromatic foci, flatness of morphology and chromatin alterations.



Many pathways lead to senescence, and the latest research points to microRNAs playing a key role. Adapted from Gorospe M, Abdelmohsen K. MicroRegulators come of age in senescence. Trends Genet. 2011; 27(6):233-41.

Did you know?

Senescence is defined as the state of being old, or the process of becoming old. In plants, it is the phase from full maturity to death, as seen with leaves.

It refers not only to being or becoming old, but also the deterioration with age.

In terms of cellular senescence, it is the stage at which cells lose their ability to grow and divide, which can occur due to damage or as a result of loss in molecular components.

New Publications

- Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. Genes Dev. 2014; 28(2):99-114.
- Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dollé ME, Hoeijmakers JH, de Bruin A, Hara E, Campisi J. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell. 2014; 31(6):722-33.
- Ido Y, Duranton A, Lan F, Weikel KA, Breton L, Ruderman NB. Resveratrol Prevents Oxidative Stress-Induced Senescence and Proliferative Dysfunction by Activating the AMPK-FOXO3 Cascade in Cultured Primary Human Keratinocytes. PLoS One. 2015; 10(2):e0115341.

We are a lot like our cells...

Just like our cells get tired of replicating and senesce, as we get older, that afternoon nap starts looking really attractive.

The Hallmark & Disease

Cellular senescence has become an increasingly important target in the development of novel therapeutics. Emerging data implicates senescence bypass in the development of cancer and suggests that senescence may represent a tumor suppressor mechanism. The demonstration that tumor cells can be induced to undergo replicative senescence following the introduction of negative cell-cycle regulators, anti-telomerase peptides, or drug treatment suggests that induction of senescence can be exploited as a basis for cancer therapy.

Did you know?

Senescent cells can be distinguished from non-senescent cells base on their defined phenotype. Senescent cells show increased size, a flat morphology, lipofuscin granule accumulation, and senescence-associated β-galactosidase (SA-β-gal) activity.

Cellular Senescence Detection

Featured Technique:

Proliferation Assays

Evaluation of cell proliferation is essential for studies of most biological processes and for many cell-based assays. The traditional method for detection of cell proliferation has been the measurement of [3H] thymidine incorporation as cells enter S phase, and subsequent quantification of [3H] thymidine, as performed by scintillation counting. This technology is slow, labor-intensive and has several limitations, including the handling and disposal of radioisotopes and the necessity of expensive equipment. EMD Millipore has developed multiple technologies for biomolecular detection and cellular analysis that offer significant advantages over [3H] thymidine incorporation for quantifying cell proliferation with speed, precision, and accuracy. These include the use of non-radioactive reagents such as EdU, BrdU, WST-1, and MTT.

Featured Solution:

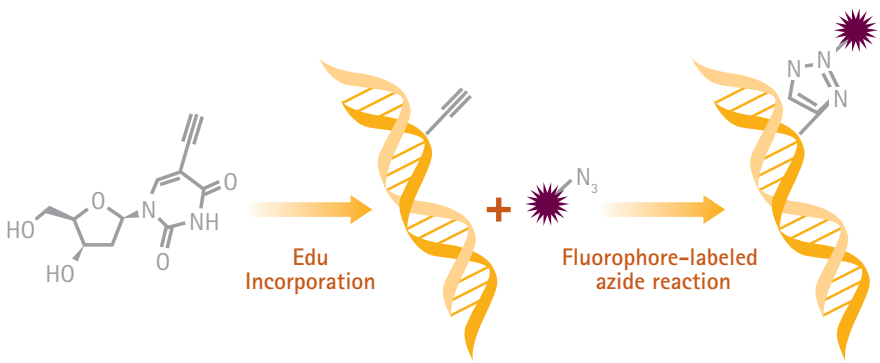
NEW EdU Cell Proliferation Assays

(Catalogue Nos. 17-10525, 17-10526, 17-10527, 17-10528)

The use of EdU (5-ethynyl-2'-deoxyuridine) as a thymidine nucleoside analog is a significant improvement compared to the classical BrdU and 3[H] thymidine cell proliferation assays. In contrast to BrdU assay kits, the EdU cell proliferation assays are not antibody based and do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the EdU Cell Proliferation assays measure the incorporation of EdU into newly synthesized DNA with click chemistry for detection by fluorescence microscopy and flow cytometry applications.

Features and Benefits

- Fast, simple workflow
- No DNA denaturation step
- No antibody
- Structurally preserves sample
- Fast detection procedure (~30 minutes)
- Compatible with flow cytometry and microscopy
- Kits available with a variety of high resolution fluorescent readouts (EdU488, 555, 594, 647)



EdU Cell Proliferation Assay Principle. Cells grown in the presence of 5-EdU incorporate the compound at thymidine bases during S-phase. Fluorophore-labeled azide reacts with the incorporated EdU to allow detection by microscopy or flow cytometry.

Solutions for your Research

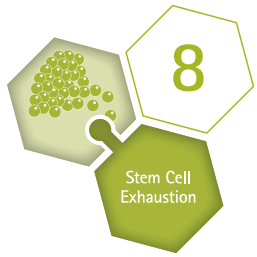
EMD Millipore offers effective solutions for research on topics related to Cellular Senescence:

Research	Description	Cat. No.
Senescence	Cellular Senescence Assay	KAA002
	Senescence Detection Kit	QIA117-1KIT
Cell Proliferation	EdU Cell Proliferation Assay (EdU-488)	17-10525
	EdU Cell Proliferation Assay (EdU-555)	17-10526
	EdU Cell Proliferation Assay (EdU-594)	17-10527
	EdU Cell Proliferation Assay (EdU-647)	17-10528
	Cell Proliferation Assay Kit, WST dye; ELISA based	2210
	BrdU Cell Proliferation Kit	2750, 2752
	Calbiochem® BrdU Cell Proliferation Assay	QIA58
Growth and Viability	MTT Cell Growth Assay Kit	CT01
	MTT Cell Growth Assay Kit	CT02
	Muse® Count & Viability Assay Kit	MCH100102
	Guava® Cell Growth Kit for Flow Cytometry	4500-0270
Cytokines and Growth Factors	Fibroblast Growth Factor basic Protein, Human recombinant	GF003
	Transforming Growth Factor-β1 Protein, Recombinant human	GF111
	Hepatocyte Growth Factor Protein, Recombinant human	GF116
	Insulin-like Growth Factor-I Protein, Recombinant human	GF138
	Insulin-like Growth Factor-I Protein, Recombinant human	GF140
Cell Culture Media and Supplements	Fetal Bovine Serum, US Origin	TMS-013-B
	EmbryoMax® ES Cell Qualified Fetal Bovine Serum	ES-009-B
	AOF ITS Supplement (100X), 10 mL	SCM054
	EmbryoMax® DMEM (1X), liquid, With 4,500mg/L Glucose, 2.25g/L Sodium Bicarb and L-Glut, without Sodium Pyruvate	SLM-120-B
	EmbryoMax® DMEM/F12, with L-Glutamine, without HEPES	DF-042-B
	EmbryoMax® MEM, Non Essential Amino Acids (100X)	TMS-001-C
	EmbryoMax® L-Glutamine Solution (100X), 200mM	TMS-002-C
Primary Cells and Cell Lines	EndoGRO® Human Umbilical Vein Endothelial Cells (HUVEC)	SCCE001
	EpiGRO® Human Ocular Epithelia Complete Media Kit	SCMC001
	FibroGRO® Xeno-Free Human Foreskin Fibroblasts	SCC058

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

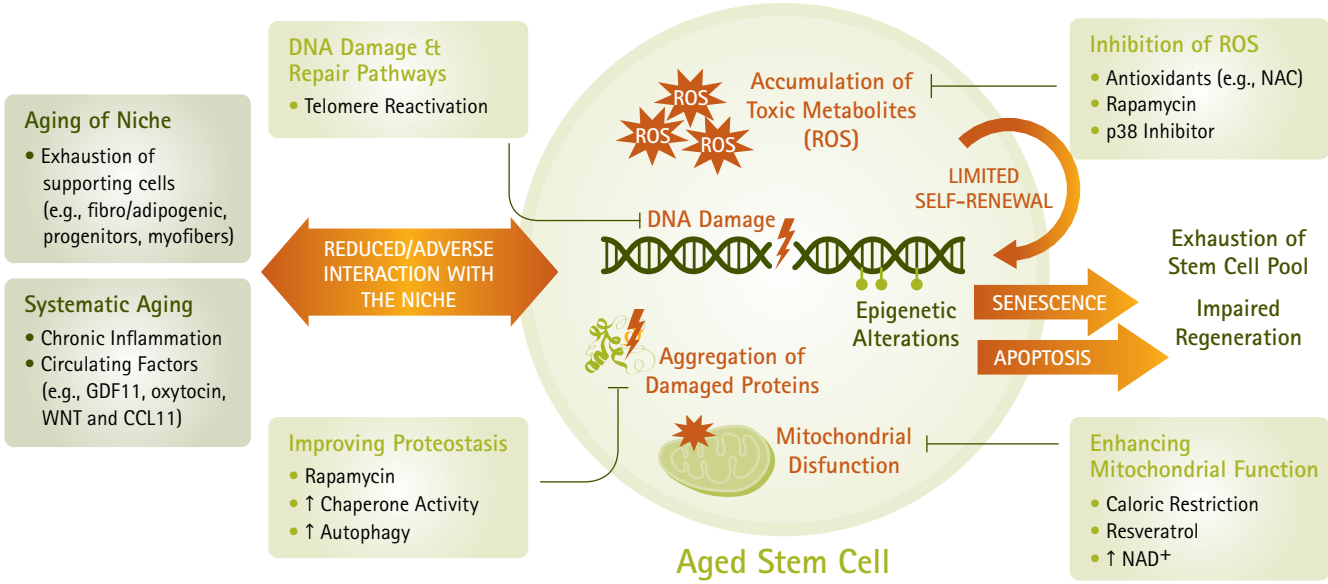
Notes

Stem Cell Exhaustion



As we age, our stem cells eventually lose their ability to divide. Furthermore, we are unable to replace the stem cells that have migrated, differentiated, or died. As a result, we show outward symbols of aging, such as grey hair.

While the decrease in the renewal of stem cells certainly leads to age-related disorders, it is clear that this "stem cell exhaustion" is really a consequence of DNA damage, deregulated nutrient sensing, senescence, and other processes already mentioned—in other words, it might be argued that it is not a "true" hallmark. Nevertheless, because of their unique role in determining cell fate in a tissue-specific way, stem cells can reveal ways that tissues interact during the aging of a complex organism and possibly redirect the fate of aging tissues upon transplantation.



Hallmarks of aging affect aging stem cells. Adapted from Oh J, Lee YD, Wagers AJ. Stem cell aging: mechanisms, regulators and therapeutic opportunities. Nat Med. 2014 Aug;20(8):870-80.

Recent studies have asked how environmental, genetic and microenvironmental factors all work together to affect stem cell fate. "Youthful" signals (like microRNAs) can apparently be delivered to aging stem cells via extracellular vesicles—could these vesicles serve as anti-aging therapeutics?

It's also clear that diet and metabolic signaling also affects stem cells, as do signals from the microbiome. The discovery of Toll-like receptors on intestinal stem cells points to a paradigm in which our aging is determined not only by what we eat and breathe, but also the bacteria we carry.

Did you know?

Grey hair are the result of depletion of stem cells in the hair follicles. Interestingly, it has been found that the loss of melanocyte stem cells from hair follicles also results from stress.

New Publications

- Walter D, Lier A, Geiselhart A, Thalheimer FB, Huntscha S, Sobotta MC, Moehrle B, Brocks D, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015; 520(7548):549-52.
- Pusic AD, Kraig RP. Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. Glia. 2014; 62(2):284-99.
- Mihaylova MM, Sabatini DM, Yilmaz ÖH. Dietary and metabolic control of stem cell function in physiology and cancer. Cell Stem Cell. 2014; 14(3):292-305.

We are a lot like our cells...

Just like our stem cells get depleted, as we age so does our ability to produce black hair. Our hair turns gray because melanocyte stem cells die, via pathways also linked to melanoma. Gray hair's a small price to pay for tumor suppression, so thank the next gray hair you find.

The Hallmark & Disease

Hutchinson–Gilford progeria syndrome (HGPS), also known as progeria, is a segmental premature aging disease that is caused by the exhaustion of stem cells, which inhibits the capacity for tissues to repair and regenerate. Progerin is a key protein involved in HGPS, and it has been found that progerin expression initiates differentiation in human mesenchymal stem cells, leading to their depletion. A *de novo* single nucleotide mutation in exon 11 in the LMNA gene results in the production of the defective protein progerin, and causes 90% of HGPS cases.

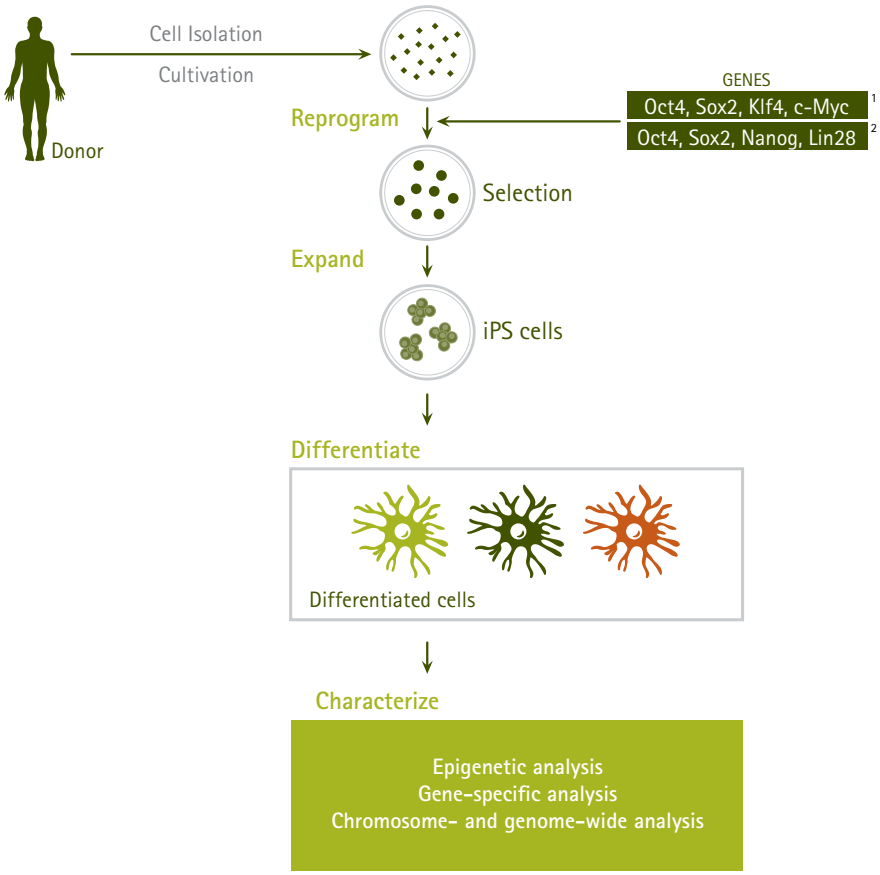
Stem Cell Research

Featured Technique:

Developing *In Vitro* Stem Cell Models

The discovery that somatic cells could revert back to pluripotent like cells (induced pluripotent stem cells) in 2006 by Shinya Yamanaka created an entirely new area of stem cell biology. IPS cells removed the ethical concerns associated with embryonic stem cells and allowed scientists to model human diseases that were previously impossible to model such as Alzheimer's, Parkinson's and autism. The current science of inducing pluripotency in cells has yielded practical technologies and protocols for a new generation of applied stem cell research. These innovations have advanced all steps of the reprogramming workflow: iPS cell generation; iPS cell culture; iPS cell characterization; and iPS cell differentiation. iPS cell clones must be carefully characterized before any application in diagnosis or therapy. Following characterization of ES-like state, the iPS cells can be guided down distinct differentiation pathways using various growth factors, small molecules, or other extracellular microenvironment manipulations.

EMD Millipore is dedicated to developing and refining these induced pluripotent stem (iPS) cell technologies. With EMD Millipore's comprehensive portfolio of kits, reagents, media, and supplements, researchers now have reliable, high-quality solutions for cellular reprogramming.



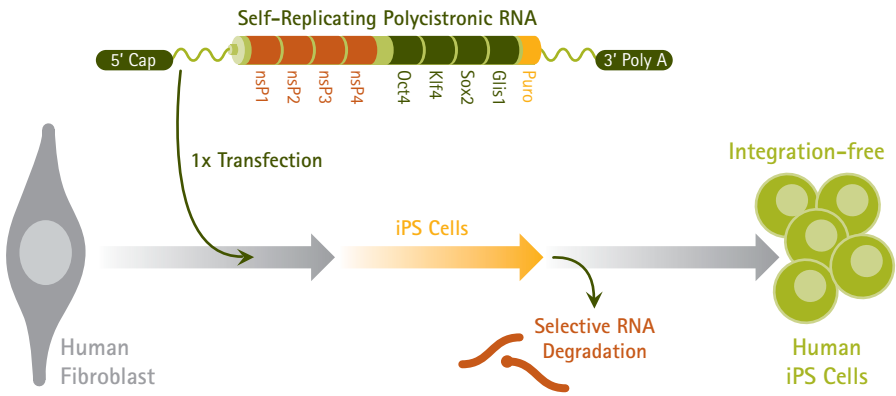
Featured Solutions:

Simplicon® RNA Reprogramming Kit

(Catalogue No. SCR550)

EMD Millipore's Simplicon® RNA Reprogramming Kit is a safe and efficient method to generate integration free, virus-free human iPS cell using a single transfection step. The technology is based upon a positive strand, single-stranded RNA species derived from non-infectious (non-packaging), self-replicating Venezuelian equine encephalitis (VEE) virus. The Simplicon® RNA replicon is a synthetic in vitro transcribed RNA expressing all four reprogramming factors (OKS-iG; Oct4, Klf4, Sox2, and Glis1) in a polycistronic transcript that is able to self-replicate for a limited number of cell divisions.

How Simplicon® Technology Works

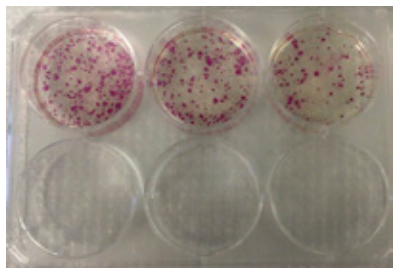


Did you know?

The nuclear lamina plays key roles in DNA replication, transcription, and repair. The LMNA gene encodes the A-type lamins, major proteins of the nuclear lamina. The mutation in LMNA results in the production of a truncated mRNA transcript encoding a prelamin A protein, known as progerin. This internal deletion alters the posttranslational processing of prelamin A and has serious effects on multiple cellular functions, leading to the phenotypes typical of progeria. Cells show nuclear blebbing, thickened nuclear lamina, abnormal distribution of nuclear pore complexes, and loss of peripheral heterochromatin.

Advantages of the Simplicon® RNA Reprogramming Kit:

- Integration-free, footprint-free iPS cells. No risk of genomic integration
- Safe, virus free, synthetic polycistronic RNA replicon (all four reprogramming factors in 1 RNA strand)
- Only 1-day transfection required. The RNA replicon is able to self-replicate, eliminating the need for laborious daily transfection of multiple individual mRNAs over a 14 day period.
- Efficient and rapid reprogramming.
- No screening required to ensure viral remnants are not present.
- Controlled elimination of synthetic RNA replicon by removal of B18R protein.
- Validated for reprogramming in feeder-based and feeder-free culture conditions.



Simplicon® RNA Reprogramming Kit. Alkaline phosphatase staining of day 28 reprogrammed BJ fibroblasts generated using Simplicon® RNA Reprogramming Technology.

PluriSTEM® Human ES/iPS Cell Medium

(Catalogue No. SCM130)

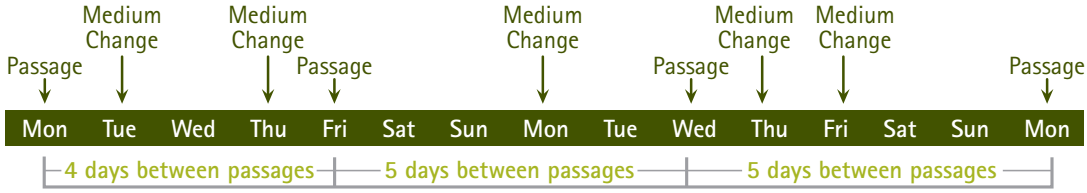
EMD Millipore's PluriSTEM® Human ES/iPS Medium is a specially formulated to maintains human pluripotent stem cells in feeder-free and serum-free conditions with less frequent feeding and cell culture time. The proprietary formulation uses Activin-A, TGFβ1 and b-FGF to promote stem cell self-renewal and potent small molecule combinations to inhibit unwanted spontaneous differentiation along with Human Serum Albumin (HSA) to aid in overall colony morphology.

PluriSTEM® Human ES/iPS Medium is provided as a stand-alone 500 mL bottle that is ready-to-use and does not require additional supplementation. Cells proliferate faster in PluriSTEM® as compared to the same cells plated in other serum-free and feeder-free media systems; a typical 4-6 day passage is expected for most pluripotent cell lines.

Feed less. Discover more.

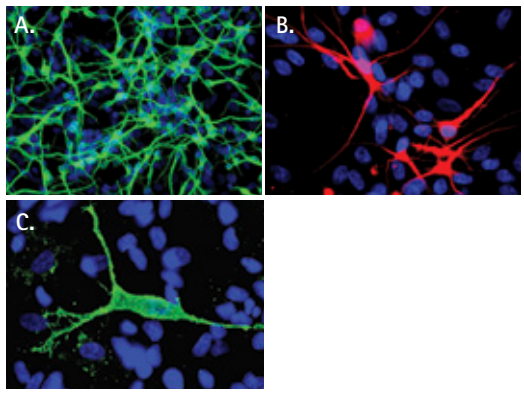
- Feeder-Free, Serum-Free, Defined Cell Culture System for Human ES/iPS Cells
- Less Frequent Cell Feeding (Every other day, weekend free) Reducing Cost and Overall Cell Culture Time
- Low Total Protein and Growth Factor Concentrations
- High Viability and Proliferation in Single Cell Passaging
- Maintains Pluripotent Stem Cells in 3D Suspension Culture

Every other day medium change and skip weekends!



ReNcell® Human Neural Stem Cells

Breakthrough studies have recently rejected the longstanding belief that neuronal tissue is incapable of regeneration. Successful engraftment of NSCs following implantation into the brain of rodent models has demonstrated the potential of this cell type in the development of regenerative therapeutic strategies. However, neural stem cells have historically proven to be difficult to isolate and culture in vitro for an extended period of time. EMD Millipore offers novel, ready-touse, neural progenitor cells isolated from both human and rodent model systems, including serum-free cell culture expansion media, and kits for differentiation and characterization.



Multipotentiality of ReNcell® cells. Both ReNcell® CX and ReNcell® VM cell lines are readily differentiated into all three neural phenoytpes: neurons (βIII-tubulin, green, 20X, A); astrocytes (GFAP, red, 40X, B) and oligodendrocytes (Gal C, green, 60X, C); all counterstained with Hoechst nuclear stain (blue).

ReNcell® immortalized human neural stem cells can readily differentiate into neurons and glial cells.

Two lines are available:

- ReNcell® VM (derived from the ventral mesencephalon region of human fetal brain tissue).
- ReNcell® CX (derived from the cortical region of human fetal brain tissue).

Solutions for your Research

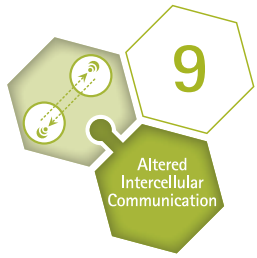
EMD Millipore offers effective solutions for Stem Cell research:

Research	Description	Cat. No.
Reprogramming Kits	Simplicon® Reprogramming RNA Kit	SCR550
	Human STEMCCA™ Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SSCR545
	Human STEMCCA™ Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	SCR544
	Mouse iPS Reprogramming Boost Supplement	SCM087
	Human iPS Reprogramming Boost Supplement II	SCM094
Stem Cell Markers & Characterization	ES Cell Characterization Kit	SCR001
	ES Cell Marker Sample Kit	SCR002
	Alkaline Phosphatase Detection Kit	SCR004
	Quantitative Alkaline Phosphatase ES Characterization Kit	SCR066
	iPS Selection Kit	SCR502
Stem Cells & Lines – Rodent	EmbryoMax® Embryonic Stem Cell Line - Strain C57/BL6, passage 11, normal male genotype	CMTI-2
	MilliTrace™ Nanog GFP Reporter Mouse Embryonic Stem Cell Kit	SCR089
	MilliTrace™ Constitutive GFP Reporter Mouse Embryonic Stem Cell Kit	SCR082
	Rat Mesenchymal Stem Cell Kit	SCR026
	N27 Rat Dopaminergic Neural Cell Line	SCC048
	O9-1 Mouse Cranial Neural Crest Cell Line	SCC049
Stem Cells & Lines – Human	ReNcell® CX Human Neural Progenitor Cell Line	SCC007
	ReNcell® VM Human Neural Progenitor Cell Line	SCC008
	Human Oligodendrocyte Differentiation Kit	SCR600
	Human Mesenchymal Stem Cells (Bone Marrow)	SCC034
	Human Adipose Mesenchymal Stem Cells	SCC038
Transfection & Reagents	GeneJuice® Transfection Reagent	70967
	NanoJuice® Transfection Kit	71902
	RiboJuice™ siRNA Transfection Reagent	71115
Growth Factors	Fibroblast Growth Factor basic Protein, Human recombinant	GF003
	HumanKine® Thermostable bFGF, Human Recombinant	GF446-10ug
	Epidermal Growth Factor Protein, Human recombinant	GF144
	HumanKine® Activin A, Human Recombinant Xeno-Free	GF400
	Transforming Growth Factor-β1 Protein, Recombinant human	GF111

For a complete selection, visit: **www.emdmillipore.com/stemcells**

Notes

Altered Intercellular Communication

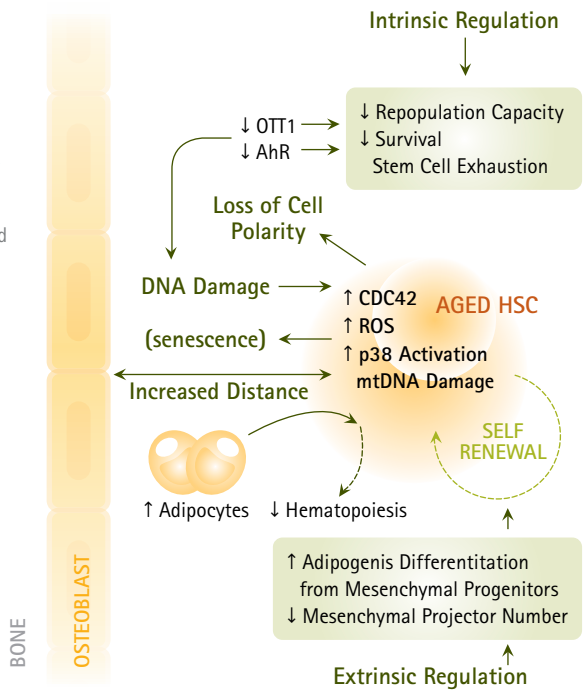


Cells, as they age, show an increase in self-preserving signals that result in damage elsewhere. Altered intercellular communication with aging contributes to decline in tissue health.

Like the decline in stem cell renewal, the age-dependent changes in intercellular communication are integrated effects of the other hallmarks of aging. In particular, senescent cells trigger chronic inflammation that can further damage aging tissues.

The cdc42 GTPase pathway, in addition to the NF-κB pathway, has been shown to increase inflammation in senescent cells; in fact, knocking down cdc42 expression actually increases longevity in *C. elegans*. GTPases also integrate signals from cell-cell junctions, which may break down in aging tissues.

At an organ system level, the aging hypothalamus drives changes in neurohormone signaling, which in turn affects food intake and metabolism. Since the hypothalamus also regulates sleep cycles, these changes can inhibit DNA repair, exacerbating the aging phenotype.



Altered signaling in aging hematopoetic stem cells (HSCs). Cdc42 and other pathways mediate signaling between aging HSCs and their niche (within the bone marrow). As HSCs age, they move further from the niche, loses cell polarity, and undergoe DNA damage.

Did you know?

Inflammaging is defined as a chronic, low-grade inflammation that is seen with aging.

Inflammaging differs significantly from acute inflammation in that it is low-grade, controlled, asymptomatic, and persistent or chronic.

Most age-related diseases share an inflammatory pathogenesis, making inflammaging a significant risk factor in our health as we age.

New Publications

- Ito TK, Yokoyama M, Yoshida Y, Nojima A, Kassai H, Oishi K, Okada S, Kinoshita D, Kobayashi Y, Fruttiger M, Aiba A, Minamino T. A crucial role for CDC42 in senescence-associated inflammation and atherosclerosis. PLoS One. 2014; 9(7):e102186.
- Paul C, Robaire B. Impaired function of the blood-testis barrier during aging is preceded by a decline in cell adhesion proteins and GTPases. PLoS One. 2013; 8(12):e84354.
- Hunt NJ, Rodriguez ML, Waters KA, Machaalani R. Changes in orexin (hypocretin) neuronal expression with normal aging in the human hypothalamus. Neurobiol Aging. 2015; 36(1):292-300.

We are a lot like our cells...

Just like our cells start sending mixed signals to other cells, as we age, we can't always send and receive signals clearly. Aging humans are lucky – we can get hearing aids to prevent miscommunication.

The Hallmark & Disease

Weakening of bones through bone loss and osteoporosis are seen in the aging population. The cause of this has been associated with chronic inflammation seen with aging. Wnt4 overexpression has been demonstrated to prevent chronic inflammation by inhibiting NF-κB activation, and also prevent bone loss. This suggests that Wnt4 signaling may be an attractive target for therapies aimed at treating osteoporosis and preventing aging related bone loss.

Did you know?

Aging affects the phenotype and functions of cells of immune system. Altered expression of innate immunity receptors or changes in their function and signal transduction leading to defective activation and decreased chemotaxis, phagocytosis, and intracellular killing of pathogens.

Cellular Communication Research

Featured Technique:

Small GTPase Signaling

Small GTP binding proteins (GTPases) function as molecular switches that regulate a number of biological processes including cell proliferation, apoptosis, differentiation, cytoskeletal reorganization, and membrane trafficking. These proteins that are active when bound to GTP and inactive when bound to GDP. Activation of small G-proteins is mediated by GDP/GTP exchange factors (GEFs) and deactivation by GTPase activating proteins (GAPs). When small GTPases are in the active state, they interact with one or more effector molecules, which in turn stimulate downstream signaling cascades. To measure activation of small GTPases, researchers traditionally use bead based pull-down assays to isolate the active (GTP bound) form of the enzyme, followed by Western blot analysis.

Featured Solution:

Small GTPase Activation Assays

The Small GTPase Activation Kits provide a quick and easy method for detecting active small GTPases. These assays isolate the active (GTP-bound) form of the protein with the binding domains of their downstream effectors immobilized on agarose beads. The isolated active GTPase is then detected by Western blot using a GTPase specific antibody. Complete kits include GTPase binding domain coupled to agarose beads, primary antibody, GDP, GTPγS, and buffer necessary to perform 20 or 30 pull-down assays.

GTPase Binding Domain	Activation Assay	Cat. No.
Pak1 p21-binding domain (PAK1-PBD)	Rac Activation Kit	17-283
	Rac1/Cdc42 Activation Kit	17-441
	Rac2 Activation Assay Kit	17-369
	Cdc42 Activation Assay Kit	17-286
Rhotekin Rho-binding domain (Rhotekin-RBD)	Rho Activation Assay Kit	17-294
Raf1 Ras-binding domain (Raf1-RBD)	Ras Activation Assay Kit	17-218
RalGDS Rap1-binding domain (RalGDS-RBP)	Rap1 Activation Assay Kit	17-321
Ral binding protein 1 (RalPB1-RBD)	Ral Activation Assay Kit	17-300
	RalB Activation Assay	17-439

Solutions for your Research

EMD Millipore offers effective solutions for research on topics related to Intercellular Communication:

Research	Description	Cat. No.
Small GTPase	Rac/Cdc42 Assay Reagent (PAK1-PBD, agarose)	14-325
	Rho Assay Reagent (Rhotekin-RBD, agarose)	14-383
	Rac1 Activation Magnetic Beads Pulldown Assay	17-10393
	Rac1/Cdc42 Activation Magnetic Beads Pull Down Assay	17-10394
	Anti-Cdc42 Immunoblotting Kit	17-299
	B-Raf Kinase Assay Kit, Chemiluminescence Detection	17-359
	Raf-1 Kinase Assay Kit, Chemiluminescence Detection	17-360
	Ras Activation ELISA Assay Kit	17-497
	Rho-associated Kinase (ROCK) Activity ELISA Assay	CSA001
Transcription Factor Assays	Universal EZ-TFA™, Colorimetric	70-500, 70-501
	Universal EZ-TFA™, Chemiluminescent	70-600, 70-601
	EZ-TFA™ NFκB p50/p65, Colorimetric	70-510
	EZ-TFA™ NFκB p50, Colorimetric	70-515
	EZ-TFA™ NFκB p65, Colorimetric	70-520
	EZ-TFA™ NFκB Family, Colorimetric	70-560
	EZ-TFA™ NFκB p50, Chemiluminescent	70-615
Cell Migration, Invasion, Angiogenesis, Vascular Permability Kits and Assays	QCM™ High Sensitivity Non-cross-linked Collagen Invasion Assay, 24-well (8 µm), Colorimetric	ECM1401
	QCM™ Chemotaxis Cell Migration Assay, 24-well (8 µm), Colorimetric	ECM508
	QCM™ Chemotaxis Cell Migration Assay, 96-well (8 µm), Fluorimetric	ECM510
	QCM™ ECMatrix Cell Invasion Assay, 24-well (8 µm), Colorimetric	ECM550
	QCM™ ECMatrix Cell Invasion Assay, 24-well (8 µm), Fluorimetric	ECM554
	Cell Comb™ Scratch Assay	17-10191
	In Vitro Angiogenesis Assay Kit	ECM625
	Fibrin *In Vitro* Angiogenesis Assay	ECM630
	In Vitro Vascular Permeability Assay (96-well)	ECM642
	In Vitro Vascular Permeability Assay (24-well)	ECM644

For a complete selection, visit: **www.emdmillipore.com**

Notes

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Hallmarks of Aging

Research Solutions Guide

What's inside...

Nine chapters, one for each Hallmark, with:

- An introduction to the hallmark
- Informative illustrations
- Related new publications
- *Did you know?* (informational bits)
- *We are a lot like our cells...* (the lighter side)
- *The Hallmark & Disease*
- Featured Technique
- Featured Solution(s)
- Effective Solutions to advance your Hallmark Related Research

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