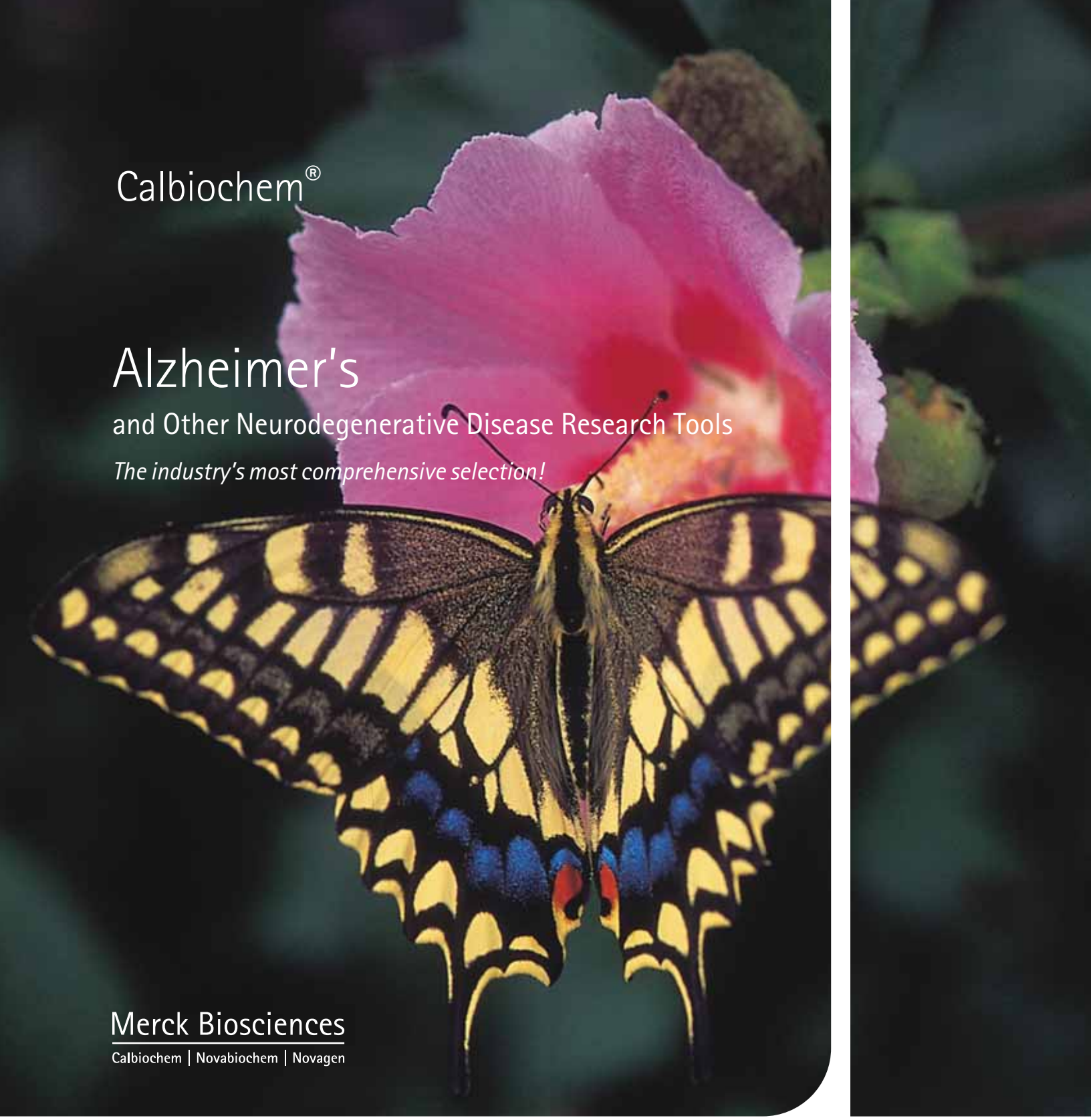

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Alzheimer's Disease:
The Role of β-Amyloid Peptides & Oxidative Stress

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Alzheimer's disease (AD), the principal cause of senile dementia, is characterized by regional neuronal degeneration, synaptic loss, and the presence of neurofibrillary tangles (NFTs) and senile plaques. NFTs are aggregates of hyperphosphorylated microtubular Tau protein, whereas the senile plaques are complex extracellular lesions composed of a β-amyloid-(Aβ)-containing core that is surrounded by activated microglia, fibrillary astrocytes, and dystrophic neurites. A decade of research has established that reactive oxygen species (ROS) contribute extensively to the neuronal damage in AD. Oxidative damage is probably one of the early markers of neuronal dysfunction in AD. With advancing age there is increased production of ROS and diminished capacity to protect against ROS, leading to an increased oxidizing cellular environment. A strong correlation is reported to exist between the extent of free radical generation by Aβ and neurotoxicity. In addition to its direct neurotoxic effects, Aβ may also fragment into free radical peptides (containing 25 - 35 amino acids) that act as potent initiators of lipid peroxidation.

Deposition of Aβ is an early event in the pathogenesis of AD that precedes the formation of Tau-positive paired helical filaments (PHFs) in NFTs. AD is also characterized by a progressive deposition of the Aβ peptide in senile plaques. In normal healthy individuals, Aβ peptides are present only in small quantities as soluble monomers that circulate in cerebrospinal fluid and blood. In AD patients, however, their levels increase significantly and they begin to accumulate as insoluble, fibrillar plaques. The Aβ in senile plaques vary in length from 40 to 43 amino acids, however, $Aβ_{1–42}$ occurs more frequently and forms fibrillar aggregates far more readily than the $Aβ_{1–40}$ peptide. Aβ peptides originate from the proteolytic cleavage of the amyloid precursor protein (APP). The β-amyloid gene, located on chromosome 21, encodes the transmembrane APP. APP is reported to occur in three common isoforms, APP695, APP751, and APP770. The APP695 is expressed exclusively in neurons, whereas APP751 and APP770 are present in both neural and non-neural cells. The primary structure of APP contains a small signal sequence, a large extramembranous N-terminal region, a single transmembrane domain, and cytoplasmic C-terminal tail.

Processing of APP *in vivo* occurs by two major pathways. Cleavage of APP at the N-terminus of the Aβ region by β-secretase and at the C-terminus by γ-secretases represents the amyloidogenic pathway for processing of APP (See figure on page 2). The β-secretase cleaves APP between residues Met^{671} and Asp^{672} and yields sAPPβ and C99. The β-secretase has also been identified as an aspartyl protease (BACE or Asp-2) of unusual nature. It has a C-terminal transmembrane domain

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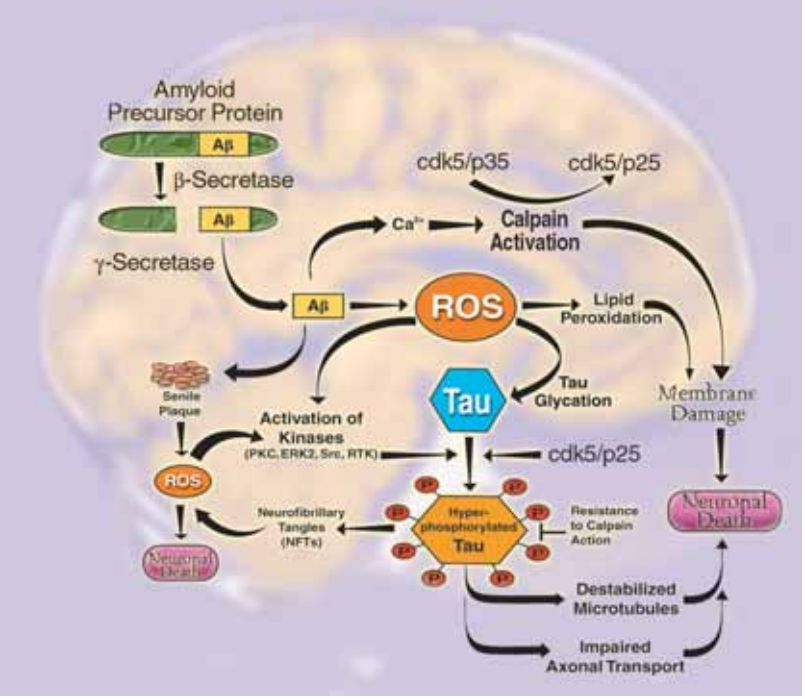

and two active site motifs located in the luminal domain. Newly synthesized BACE contains a propeptide domain, which is cleaved at residue E46 to produce the mature enzyme. The active site of BACE and the β-secretase cleavage site of APP are in precise topological orientation for endoproteinases. Succeeding the β-secretase cleavage, a second cleavage occurs at the C-terminus of Aβ peptide that releases Aβ from C99. This cleavage occurs in the vicinity of residue 712 of the C-terminus. The γ-secretase can cleave the C-terminal region at either Val^{711} or Ile^{713} to produce the shorter Aβ peptide ($Aβ_{1-40}$) or the longer Aβ peptide ($Aβ_{1-42}$). The predominant form of Aβ found in the cerebrospinal fluid is the shorter Aβ40 peptide. Despite its lower rate of synthesis, $Aβ_{1–42}$ is the peptide that is initially deposited within the extracellular plaques of AD patients. In addition, $Aβ_{1–42}$ is shown to aggregate at a much lower concentration than the $Aβ_{1–40}$ form.

APP can also be processed by α-secretase, which cleaves within the Aβ domain between Lys^{687} and Leu^{688} and produces a large soluble α-APP domain and the C-terminal fragment containing P3 (C83). The latter can then be cleaved by γ-secretase at residue 711 or 713 to release the P3 fragment. This pathway does not yield Aβ peptide. Hence, shunting APP towards the α-secretase pathway may have a beneficial effect in lowering Aβ peptide levels. It is reported that α-secretase shares many of its properties with the secretase that cleaves angiotensin-converting enzyme and is believed to be a zinc metalloproteinase of the ADAMs family. Muscarinic agonists (M1 and M3) and some PKC activators are reported to enhance α-secretase activity and are under consideration for their therapeutic value as AD treatment tools.

Neuronal toxicity to Aβ occurs via several different mechanisms, of which free radical induced damage appears to the most prominent one. Aβ can activate inflammatory pathways by enhancing the microglial secretion of inflammatory cytokines, such as IL-1 and IL-6. Additionally, it can trigger the production of ROS, nitrogen intermediates, and TNF-α from microglia. Aβ also increases the accumulation of H_2O_2 in a Cu^{2+}/Zn^{2+}-dependent manner, which can lead to free radical-induced lipid peroxidation and cell death. *In vitro*, $Aβ_{1–42}$ is shown to induce apoptosis in cultured cortical neurons, possibly through alterations of cellular calcium homeostasis. Interaction between Aβ and ApoE3 or E4 is considered to be an important determinant of amyloidosis. ApoE3 is shown to inhibit Aβ aggregation *in vitro* by decreasing Aβ multimers, whereas ApoE4 is reported to accelerate the rate of amyloid fibril formation (Ab_{1-42} > Ab_{1-40}).

Another set of proteins, known as presenilins (PS1 and PS2), are also reported to play an important role in APP processing. They are tightly linked to γ-secretase mediated cleavage. Mutations in presenilin, PS1 and PS2, genes are reported to enhance amyloid deposition. PS1 has been suggested to have either an inherent γ-secretase activity or act as a co-factor for γ-secretase. Studies of Li et al. have indicated that the active site of γ-secretase is shared between the N- and C-terminal fragments of presenilin. Cells obtained from PS1/PS2 double knockout mice do not show any γ-secretase activity. Presenilins are also involved in the regulation of Notch signaling that is important in framing cell destiny during embryogenesis, hematopoiesis, and neural stem cell differentiation. PS1 also plays an important role in the formation of the axial skeleton and in neurogenesis and survival of progenitor cells and neurons in specific brain regions.

In AD patients all mutations in APP are shown to increase $Aβ_{1–42}$ production. Most cases of familial AD are reported to result from mutations

in one of the three genes, APP, PS1 and PS2. Any mutation in these genes results in elevated levels of Aβ peptide. The mutation in APP gene, located on chromosome 21, accounts for about 2% of all cases of familial AD (FAD) and approximately 5 - 20% of early-onset FAD. A substitution of Glu to Gln at codon 693 of APP is termed "Dutch mutation," which is responsible for hereditary cerebral hemorrhage with amyloidosis (Dutch type). Here amyloid deposits containing the Aβ peptide are found in cerebral vessel walls with diffuse plaques in the brain parenchyma. Another mutation known as "Flemish mutation" occurs at codon 692 and where Ala is replaced by Gly. It causes an intermediate phenotype between congophilic angiopathy and AD. A well-studied mutation, "Swedish mutation," results from the replacement of Lys and Met by Asn and Leu at codons 670 and 671. The Swedish mutation does not lie within Aβ peptide region but lies in the proximity of the secretase cleavage sites and produces mainly the soluble $A\beta_{1-40}$ peptide. Fibroblast cell lines transfected with the Swedish mutation are shown to produce elevated levels of the soluble form of Aβ peptide. Over 40 different mutations have been reported in PS1, which account for about 30 to 50% of all presenile FAD. The PS2 gene mutations are rather rare and account for less than 2% of all early-onset FAD. Mutations in both PS1 and PS2 are associated with an increased production of the $A\beta_{1-42}$ peptide, the more amyloidogenic form of Aβ peptide. It has been suggested that mutant PS1 proteins alter the proteolytic processing of APP at the C-terminus of Aβ and favor the deposition of $A\beta_{1-42}$ peptide.

The localization of Tau protein in the AD brain is markedly abnormal and may contribute to neuronal dysfunction. In AD, normal soluble cytoskeletal elements, such as Tau and neurofilaments are transformed into insoluble PHFs. This is linked to the post-translational change in Tau, primarily the hyperphosphorylation of Tau by a number of protein kinases. Phosphorylation is intimately tied to oxidative stress via the MAP kinase pathway and through activation of NF-κB. Pyramidal neurons of the hippocampus undergoing degeneration are reported to show higher levels of free carbonyls, lipid peroxide adduction, and nitrotyrosine. Tau is a neuronal protein located mostly in the axon and, to a lesser extent in cell bodies, but is almost absent from dendrites. *In vitro*, Tau is a substrate for a multitude of protein kinases including CaM kinase II, casein kinase II, PKA, ERK2, and GSK3. Cyclin-dependent kinase 5 (Cdk5) in conjunction with its neuron-specific activator p35 (cdk5/p35) is another protein kinase implicated in Tau hyperphosphorylation. Proteolytic cleavage of the regulatory unit p35 by calpain produces p25 that accumulates in the AD brain. This cleavage is reported to be induced by Aβ in cortical neurons. Another protein kinase that has been implicated in the phosphorylation of Tau in Alzheimer's disease is MARK (microtubule regulating kinase), which preferentially phosphorylates KXGS motifs in the microtubule affinity binding domains. MARK predominantly phosphorylates Tau on Ser^{262}, although Ser^{293}, Ser^{324}, and Ser^{356} are also phosphorylated. In fact it has been suggested that phosphorylation of Ser^{262} in AD may be a primary event contributing to Tau dysfunction and, eventually, PHF and NFT formation. PHF-Tau is reported to be at least partially phosphorylated at 19 sites, of which 9 sites have a Ser/Thr-Pro motif.

A significant consequence of Tau hyperphosphorylation in AD is a reduction in its ability to bind microtubules and promote microtubule assembly. Hyperphosphorylated Tau may contribute to a destabilized microtubule network, impaired axonal transport, and ultimately in NFT formation and neuronal death. Phosphorylation of Tau at only a few sites within the microtubule-binding regions (Ser^{262}, Ser^{356}, and to a lesser extent Ser^{293} and Ser^{324}) is sufficient to diminish its ability to bind microtubules. Another interesting fact to note is that hyperphosphorylated Tau is far more resistant to degradation by calpain, a calcium-activated protease. This may be the result of Tau self-association that occurs throughout microtubule binding domains, thereby reducing the accessibility of these sites to calpain. Self-association of Tau is potentiated in an oxidizing environment. Another significant outcome of increased Tau aggregation in an oxidizing environment is glycosylation, the non-enzymatic addition of a reducing sugar to a protein. This often occurs on a lysine residue and may result in the formation of Schiff bases. PHF-Tau that is both glycated and hyperphosphorylated shows a greater reduction in microtubule-

binding capacity compared to soluble Tau from AD brain that is hyperphosphorylated, but not glycated. Oxidative cross-linking also makes proteins more resistant to proteolytic removal by inhibiting the activity of proteasomes. Therefore, oxidative cross-linking may be a significant contributor to the accumulation of ubiquitin conjugates in NFTs. Higher levels of ubiquitin have been reported in several neurodegenerative diseases.

An important question that arises is whether reducing oxidative stress has any therapeutic value in minimizing the pathogenesis of AD. Agents that inhibit free radical formation have been shown to reduce the incidence and progression of AD. Addition of antioxidants, such as propyl gallate, vitamin E, and spin traps, such as N-*tert*-butyl-α-phenylnitrone, reduces neurotoxicity in cultured cells exposed to Aβ. Vitamin E has been reported to promote hippocampal neuronal survival *in vitro* and restore hypofunctioning cholinergic neurons in rats. Another approach to minimize neuronal damage appears to be reducing or preventing the release of Aβ from APP. The characterization of the APP secretases during the past few years has provided significant advancement in therapeutic strategies that may lead to limiting the build up of Aβ peptides in the brain and eliminating or delaying the pathological effects of AD.

Inhibiting the activity of β- or γ-secretase is therapeutically attractive because clinical intervention at this step affects the early events that lead to plaque formation and neuronal death. Small molecules that inhibit β- and/or γ-secretase would therefore be expected to decrease production of Aβ and retard the progression of AD. Major focuses in AD research are to identify more genetic and environmental factors responsible for Aβ build-up in nerve cells.

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Antibodies to β-Amyloids, Amyloid Precursor Proteins, and Related Products

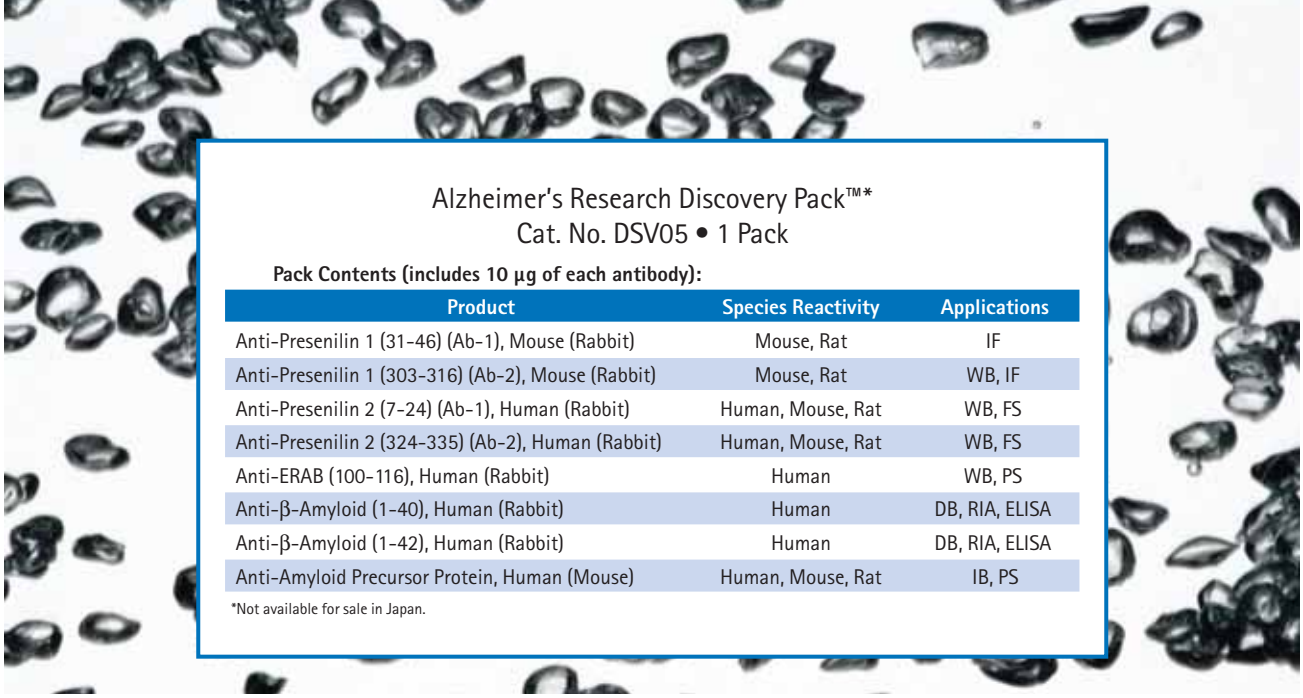
Product Name	Cat. No. / Applications	Size / Price	Comments
Anti-Amyloid Precursor Protein, N-Terminal (44-63), Human (Goat)	**171598** ELISA, FS, PS	100 µl	Immunogen used was a synthetic peptide corresponding to amino acid residues 44 - 63 of N-terminus of human APP.
Anti-Amyloid Precursor Protein, C-Terminal (681-695), Human (Goat)	**171599** ELISA, IB	100 µl	Recognizes 110 - 135 kDa human APP. Also recognizes small degraded APP products by immunoblotting.
Anti-Amyloid Precursor Protein, C-Terminal (751-770), Human (Rabbit)	**171610** IB, IF, IH, IP	50 µl	Detects C-terminal soluble products CTFγ (~6 kDa), CTFα (~9 kDa), and CTFβ (~11 kDa), and full length APP.
Anti-Amyloid Precursor Protein, Frameshift Mutant, C-Terminal (339-348), Human (Goat)	**171600** ELISA, IB, PS	100 µl	Recognizes a frameshift mutant with a GA deletion, resulting in a truncated 45 kDa APP protein with a unique C-terminus.
Anti-Amyloid Precursor Protein, Human (Mouse)*	**171537** IB, PS	100 µg	Recognizes amino acid residues 18 - 38 of human and mouse APP_{695}.
Anti-β-Amyloid Precursor-Like Protein 1, N-Terminal, Human/Mouse (Rabbit)	**NE1009** IB, IP	100 µl	Specifically recognizes APLP1. Does not react with APP and APLP2. APLP1 is a member of the APP family that, like APP, is processed in a presenilin-1 manner.
Anti-β-Amyloid Precursor-Like Protein 1, C-Terminal (643-653), Mouse, (Rabbit)	**171615** IB, IF, IP	100 µl	Recognizes specifically APLP1. Does not react with APP and APLP2.
Anti-β-Amyloid Precursor-Like Protein 2, C-Terminal (752-763), Mouse, (Rabbit)	**171616** IB, IF, IP	100 µl	Recognizes specifically APLP2. Does not react with APP or APLP1.
Anti-β-Amyloid Precursor-Like Protein 2, Full-Length, Mouse (Rabbit)	**171617** IB, IF, IH, IP	100 µl	Recognizes specifically APLP2. Does not react with APP or APLP1.
Anti-β-Amyloid (Asp-1) (FCA18), Human (Rabbit)	**PC729** IB, IP, PS	25 µl	Interacts only with the first free aspartyl- residue and recognizes the N-terminus part of $Aβ_{1-x}$. Does not recognize aspartyl- residues in full length APP or N-acetylated aspartyl- or aspartyl-1 deleted Aβ peptides. Aβ(Asp-1) is conserved between species.
Anti-β-Amyloid (1-17), Human (Mouse)	**NE1003** ELISA, IB, IP, PS	100 µl	Reacts with abnormally processed isoforms as well as precursor forms of β-amyloid.
Anti-β-Amyloid (17-24), Human (Mouse)	**NE1002** ELISA, IB, IP, PS	100 µl	Reacts with abnormally processed isoforms as well as precursor forms of β-amyloid. Weakly recognizes mouse.
Anti-β-Amyloid (1-40), Human (Rabbit)	**PC149** DB, ELISA, RIA	25 µg	Pre-absorbed against both β-amyloid (1-42) and (1-43). Specific only for $Aβ_{1-40}$.
Anti-β-Amyloid (1-42), Human (Rabbit)	**PC150** DB, ELISA, RIA	25 µg	Pre-absorbed against both β-amyloid (1-40) and (1-43). Specific only for $Aβ_{1-42}$.
Anti-β-Amyloid (1-43), Human (Rabbit)	**PC151** DB, ELISA, RIA	25 µg	Pre-absorbed against both β-amyloid (1-40) and (1-42). Specific only for $Aβ_{1-43}$.
Anti-β-Amyloid, N-Terminal, Human (Mouse)	**171603** ELISA, IB, IC	100 µg	Recognizes human β-amyloid peptide and exhibits only minor cross-reactivity with amyloid precursor protein (APP).
Anti-β-Amyloid (1-40), C-Terminal, Human (Mouse)	**171604** ELISA, IB, IC	100 µg	Immunogen used was a synthetic peptide corresponding to the C-terminus of the β-amyloid (1-40) peptide. Does not cross-react with $Aβ_{1-42}$.
Anti-$β\text{-Amyloid}_{40}$ (FCA3340), Human (Rabbit)	**171608** EIA, ELISA, IB IF, IH, IP	50 µl	Specifically recognizes $Aβ_{40}$ and p3-related fragments, and does not recognize APP. Does not cross-react with $Aβ_{42}$ and $Aβ_{43}$.

Key: DB: Dot Blot; **EIA:** Enzyme Immunoassay; **ELISA:** Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **FS:** Frozen Sections; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation: **PS:** Paraffin Sections; **RIA:** Radioimmunoassay **WB:** Western Blot

Note: *Not available for sale in Japan.

Product Name	Cat. No. Applications	Size Price	Comments
Anti-β-Amyloid (1-40/42), C-Terminal, Human (Mouse)	**171605** ELISA, IB, IC	100 µg	Immunogen used was a synthetic peptide corresponding to the C-terminal of both β-amyloid (1-40) and β-amyloid (1-42). Reacts with both $Aβ_{1-40}$ and $Aβ_{1-42}$.
Anti-β-Amyloid (1-42), C-Terminal, Human (Mouse)	**171606** ELISA, IB, IC	100 µg	Immunogen used was a synthetic peptide corresponding to the C-terminus of the β-amyloid (1-42) peptide. Does not cross-react with $Aβ_{1-40}$.
Anti-β-$Amyloid_{42}$ (FCA3542), Human (Rabbit)	**171609** EIA, ELISA, IB IF, IH, IP	50 µl	Specifically recognizes $Aβ_{42}$ and p3-related fragments, and does not recognize APP. Does not cross-react with $Aβ_{40}$ and $Aβ_{43}$.
Anti-β-Amyloid (1-43), C-Terminal, Human (Mouse)	**171607** ELISA, IB, IC	100 µg	Specifically recognizes C-terminus of β-amyloid (1-43). Does not cross-react with either $Aβ_{1-40}$ or $Aβ_{1-42}$.
Anti-Pan β-Amyloid, Human (Rabbit)	**PC152** DB, ELISA, IH, RIA	100 µg	Recognizes all three β-amyloid peptides; $Aβ_{1-40}$, $Aβ_{1-42}$, and $Aβ_{1-43}$.
Anti-BACE (Ab-1), Human (Rabbit) (Anti-β-Secretase)	**PC478** PS	50 µl	Immunogen used was a synthetic peptide corresponding to amino acid residues 44 - 59 of human BACE.
Anti-BACE (Ab-2), Human (Rabbit)	**PC529** IB	100 µg	Immunogen used was a synthetic peptide corresponding to amino acids 485 - 501 of human BACE. Reacts with human and mouse.
Anti-BACE (26-45), Human (Rabbit)	**195100** IB	100 µl	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE cDNA corresponding to BACE with an intact prodomain.
Anti-BACE (46-65), Human (Rabbit)	**195101** IB	100 µl	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE. The bands combine to a single band of ~50 kDa after treatment with endoglycosidase F.
Anti-BACE (487-501), Human (Rabbit)	**195102** IB	100 µl	Recognizes bands of ~65 and ~75 kDa in CHO cells transfected with BACE cDNA. The bands combine to a single band of ~50 kDa after treatment with endoglycosidase F.
Anti-BACE1, C-Terminal (485-501), Human (Guinea Pig)	**195110** IB	100 µl	Recognizes both the immature and mature forms of BACE1. Reacts with human, mouse, and rat.
Anti-BACE1, C-Terminal (485-501), Human (Rabbit)	**195111** IB, IF, IH, IP, RIA	100 µl	Recognizes both the immature and mature forms of BACE1. Reacts with human, mouse, and rat.
Anti-BACE2 (Ab-1), Human (Rabbit)	**PC555** IB **PC555T** IB	100 µg 10 µg	Immunogen used was a synthetic peptide corresponding to amino acids 496 - 511 of human BACE2. Cross-reacts with 52 and 39 kDa proteins to a lesser extent.
Anti-BACE2 (Ab-2), Human (Rabbit)	**PC556** IB **PC556T** IB	100 µg 10 µg	Immunogen used was a peptide corresponding to amino acid residues 44 - 59 of human BACE2. Reacts with human, mouse, and rat.
Anti-BACE2 (131-142), Human (Rabbit)	**195103** IB	100 µl	Recognizes the expected ~47 kDa band in guinea pig brain cell lysates. Also detects a ~28 kDa band not detected by the preimmune serum.
Anti-BACE2 (504-518), Human (Rabbit)	**195104** IB	100 µl	Recognizes the expected ~47 kDa band in guinea pig brain cell lysates. Also detects a ~62 kDa band not detected by the preimmune serum.
Anti-ERAB (100-116), Human (Rabbit)	**PC243** IB, PS	25 µg	Immunogen used was a synthetic peptide corresponding to amino acid residues 100-116 of human ERAB. Detects a 27 kDa protein and a 35 kDa cross-reacting band in human SK-H-SH neuroblastoma cells.
Anti-ERAB (101-119), Mouse (Rabbit)	**324918** IB	100 µl	The immune serum detects the predicted ~27 kDa band in SK-N-SH cell lysates. Also reacts with human.

Key: DB: Dot Blot; **EIA:** Enzyme Immunoassay; **ELISA:** Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **FS:** Frozen Sections; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation: **PS:** Paraffin Sections; **RIA:** Radioimmunoassay; **WB:** Western Blot



Alzheimer's Research Discovery Pack™*
Cat. No. DSV05 • 1 Pack

Pack Contents (includes 10 µg of each antibody):

Product	Species Reactivity	Applications
Anti-Presenilin 1 (31-46) (Ab-1), Mouse (Rabbit)	Mouse, Rat	IF
Anti-Presenilin 1 (303-316) (Ab-2), Mouse (Rabbit)	Mouse, Rat	WB, IF
Anti-Presenilin 2 (7-24) (Ab-1), Human (Rabbit)	Human, Mouse, Rat	WB, FS
Anti-Presenilin 2 (324-335) (Ab-2), Human (Rabbit)	Human, Mouse, Rat	WB, FS
Anti-ERAB (100-116), Human (Rabbit)	Human	WB, PS
Anti-β-Amyloid (1-40), Human (Rabbit)	Human	DB, RIA, ELISA
Anti-β-Amyloid (1-42), Human (Rabbit)	Human	DB, RIA, ELISA
Anti-Amyloid Precursor Protein, Human (Mouse)	Human, Mouse, Rat	IB, PS

*Not available for sale in Japan.

Amyloid Related Proteins and Peptides

Product Name	Cat. No.	Size Price	Comments
β-Amyloid Peptide (1-40), Human, Biotin Conjugate	PP64B	250 µg	Biotin-labeled synthetic peptide corresponding to amino acid residues 1 - 40 of the processed human amyloid peptide. This peptide is non-neurotoxic prior to a preincubation step.
β-Amyloid Peptide (1-40), Human, Fluorescent-Labeled	171591	250 µg	An N-terminally modified human $A\beta_{1-40}$ peptide labeled with 7-diethylaminocoumarin-3-carbonyl (DAC). Shares a common binding site with $A\beta_{1-40}$ (Cat. No. 171590) with 40-fold greater affinity. Ex. max ~ 430 nm; Em. max ~ 470 nm.
β-Amyloid Peptide (1-40), Rat	171593	250 µg	A major component of senile and Alzheimer's plaques. Promotes the down-regulation of Bcl-2 and enhances neuronal sensitivity to oxidative damage.
β-Amyloid Peptide (1-42), Human	PP69	250 µg	The major constituent of plaques and tangles in Alzheimer's brain.
β-Amyloid Peptide (1-42), Rat	171596	250 µg	Predominant peptide found in the brain of patients with Alzheimer's disease and Down's Syndrome. Promotes down-regulation of Bcl-2 and increases levels of Bax in neurons.
β-Amyloid Peptide (1-40, Gln^{11}), Human	171590	250 µg	A major component of senile and Alzheimer's plaques. Promotes the down-regulation of Bcl-2 and enhances neuronal sensitivity to oxidative damage.
β-Amyloid Peptide (1-40, Gly^{21}), Human	PP67	500 µg	This sequence contains the mutation associated with the Flemish variant of Alzheimer's disease.
β-Amyloid Peptide (1-40, Gln^{22}), Human	PP68	500 µg	This sequence contains the mutation associated with the Dutch variant of Alzheimer's disease.
(Pro^{18}, Asp^{21})-Amyloid β-Protein (17-21)	171592	5 mg	A pentapeptide that inhibits amyloid fibril formation *in vitro* and *in vivo*. Prevents amyloid neurotoxicity.
β-Amyloid Precursor Protein, CTF-31, Synthetic	171540	250 µg	A 31-amino acid peptide (APP 740 - 770) resulting from the proteolytic cleavage of the C-terminus of β-APP at Asp^{739} - Ala^{740} by caspases. Induces apoptosis and may be involved in the neuronal death associated with Alzheimer's disease.
β-Amyloid Precursor Protein, CTF-50, Synthetic	171545	250 µg	A 50-amino acid peptide (APP 721 - 770) resulting from the γ-secretase cleavage of the C-terminus of β-APP at Leu^{720} - Val^{721}. May also serve as one of the precursors for the generation of the toxic C31, APP (Cat. No. 171540).
β-Amyloid Precursor Protein, CTF-57, Synthetic	171550	250 µg	A 57-amino acid peptide (APP 714 - 770) resulting from the γ-secretase cleavage of the C-terminus of β-APP Ala^{713} - Thr^{714}. May also serve as one of the precursors for the generation of the toxic C31, APP (Cat. No. 171540).

Amyloid Probes and Stains

Product Name	Cat. No.	Size Price	Comments
BSB	286895	5 mg	A cell-permeable fluorescent probe that specifically binds to and labels intracellular Aβ aggregates both *in vitro* (K_i = 400 nM) and *in vivo*.
BTA-1 [2-(4′-(methylamino)phenyl) benzothiazole]	203860	10 mg	A brain-permeable, fluorescent Thioflavin-Tht; (Cat. No. 596200) derivative that exhibits high affinity for amyloid deposits (K_i = 11 nM for $A\beta_{40}$). Displays up to 50-fold higher affinity than ThT. Selectively stains cerebral plaques and cerebrovascular amyloid deposits in the brains of living PS1/APP transgenic mice.
Chrysamine G	230760	10 mg 25 mg	A lipophilic, bis-salicylic acid analog of Congo Red (Cat. No. 234610) that displays both high (K_d = 200 nM; B_{max} = 1.13 moles per mole of $A\beta_{40}$) and low (K_d = 38.77 µM; B_{max} = 23.10 moles per mole of $A\beta_{40}$) affinity binding sites for Aβ fibrils. It can cross the blood-brain barrier and serves as a useful probe for detecting Aβ aggregates. Offers protection against Aβ fibrils toxicity in PC-12 and rat hippocampal neuronal cells.
Half Chrysamine G	371977	1 mg	A "half-molecule" of Chrysamine G that offers protection against $A\beta_{25-35}$ and $A\beta_{40}$-induced neuronal death at a concentration of 0.1 - 1 µM. Shown to cross the blood brain barrier and exhibit minimal *in vivo* toxicity.
Congo Red, High Purity	234610	500 mg	A diagnostic amyloidophylic dye that specifically stains stacked β sheet aggregates. Does not bind to the non-polymerized amyloid peptide. Also blocks the accumulation of PrP^{sc} (Scrapie) prion protein.
Direct Fast Yellow	322015	1 g	An azo dye containing salicylic acid moiety that specifically binds to amyloid-like β-sheet structures and inhibits (apparent IC_{50} ~ 500 nM) the aggregation of Huntington's Disease (HD) exon 1 protein. Shown to be more lipophilic than Congo Red (Cat. No. 234610).
6-Me-BTA-2	444350	50 mg	A brain-permeable, fluorescent neutral Thioflavin-Tht; (Cat. No. 596200) derivative with high affinity for amyloid deposits (K_i = 143 nM for $A\beta_{40}$). Displays up to 6-fold higher affinity than ThT. Stains both plaques and neurofibrillary (Tau protein) in post mortem Alzheimer's disease brain.
Thioflavin T	596200	500 mg	A fluorogenic probe useful in monitoring stacked β sheet aggregates. Upon binding to amyloid fibrils, ThT shifts excitation and emission maxima.

Notch 1 Related Products

Product Name	Cat. No.	Size Price	Comments
Notch 1 Peptide, Fluorogenic	491000	1 mg	An intramolecularly quenched fluorogenic peptide that includes the third proteolytic cleavage site (Gly^{1743}~ Val^{1744}) in Notch 1. Acts as a specific substrate for measuring the cellular Notch activity.
Notch 1 Mutated Peptide, Fluorogenic	491001	1 mg	An intramolecularly quenched fluorogenic Notch 1 peptide mutated at the third proteolytic cleavage site (Gly^{1743} ~ Val^{1744} → Lys). It is cleaved to a much less extent compared to Notch 1 Peptide, Fluorogenic (Cat. No. 491000) by Notch. The proteolysis is inhibitable in the presence of MG132 (Cat. No. 474790), γ-Secretase Inhibitor II (Cat. No. 565755), and γ-Secretase Inhibitor X (Cat. No. 565771)
Anti-Notch 1, Mouse (Hamster)	491010 FC, IB, IC	50 µl	Recognizes the ~300 kDa Notch 1 as well as a ~230 kDa band believed to be the processed extracellular fragment of Notch 1.

Notch 1 Related Products, cont.

Product Name	Cat. No.	Size Price	Comments
Anti-Notch 1, Cytoplasmic Domain, Rat (Rabbit)	491011 IB	50 µg	Recognizes the ~300 kDa full-length Notch 1 and the ~120 kDa cytoplasmic fragment.
Anti-Notch 1, Extracellular Domain, Rat (Rabbit)	491012 IB	50 µl	Recognizes the ~300 kDa full-length Notch 1 and the ~180 kDa extracellular fragment.
Anti-Cleaved Notch 1 (Val^{1744}), Human (Rabbit)	ST1028 ELISA, IB, IP, PS	50 µl	Detects the ~110 kDa Notch 1 cleaved at (Val^{1744}). Notch 1 can be cleaved at this site by furin-like convertase and γ-secretase.

Amyloidogenesis Inhibitors and Related Products

Product Name	Cat. No.	Size Price	Comments
$A\beta_{42}$ Fibrillogenesis Inhibitor I (H-LPFFD-OH)	171586	5 mg	A pentapeptide designed from the central hydrophobic region of the N-terminal domain of Aβ that acts as a β-sheet breaker. Inhibits Aβ fibrillogenesis, disassembles preformed fibrils *in vitro* and prevents neuronal death induced by fibrils in cell culture.
$A\beta_{42}$ Fibrillogenesis Inhibitor II (H-RVVIA-NH_2)	171587	5 mg	A pentapeptide amide that contains C-terminal sequence of $A\beta_{42}$ and acts as a β-sheet breaker. Binds to the seeding sequence of $A\beta_{42}$ peptide and interferes with its aggregation.
$A\beta_{42}$ Fibrillogenesis Inhibitor III (AC-LPFFD-NH_2)	171588	5 mg	A brain-permeable, modified analog of $A\beta_{42}$ Fibrillogenesis Inhibitor I (Cat. No. 171586) that acts as a β-sheet breaker. Increases neuronal survival with a concomitant reduction in brain inflammation by inducing a dramatic reduction in Aβ deposition in a transgenic mouse model of Alzheimer's disease.
$A\beta_{42}$ Fibrillogenesis Inhibitor IV [Ac-LP-(NMe)-FFD-NH_2]	171589	5 mg	A modified (N-methylated amide linkage between Pro and Phe) analog of the end protected $A\beta_{42}$ Fibrillogenesis Inhibitor III (Cat. No. 171588) that acts as a β-sheet breaker with improved brain uptake and increased *in vivo* metabolic stability ($t_{1/2}$ > 24 hours in human plasma and in rat brain homogenate).
β-Amyloid Ligand	171585	1 mg	Contains the short Aβ fragment (KLVFF; $A\beta_{16-20}$) and binds stereospecifically to full-length Aβ, thus preventing its assembly into amyloid fibrils.
Clioquinol	233165	1 g	A neurotoxic antibiotic that is reported to dissolve senile plaques and reduce amyloid's ability to clump together.
Flufenamic Acid	343075	1 g	A non-steroidal anti-inflammatory drug (NSAID) that acts as a potent dose-dependent inhibitor of TNFα-induced NF-κB activation. Also reported to inhibit the formation of transthyretin amyloid fibrils.
Kaempferol	420345	25 mg	A selective topoisomerase II inhibitor. Offers protection against $A\beta_{25-35}$-induced cell death in neonatal cortical neurons. Its protective effects are comparable to that of estradiol.
Mifepristone	475838	50 mg	A cell-permeable synthetic steroid that acts as a potent antagonist of progesterone and glucocorticoid receptors. As an antioxidant, it offers neuroprotection against controlled corticalimpact (CCI) in CA1 pyramidal cells, as well as Aβ-, H_2O_2-, and glutamate-induced injury to mouse hippocampal HT22 cells.
NAP	477745	5 mg	A brain-permeable, neuroprotective, femtomolar-acting, octapeptide derived from the VIP-responsive activity-dependent neuroprotective protein (ADNP). Protects neurons against toxicity associated with oxidative stress, glucose deprivation, β-amyloid peptide, NMDA, and electric blockade. Displays anti-ischemic properties by reducing apoptotic death.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

Apolipoprotein E in Alzheimer's Disease

Apolipoprotein E (Apo E) is a polymorphic, multifunctional protein synthesized by several cell types, including liver, kidney, adipose, macrophages, and brain. As a component of chylomicrons, VLDL, and a subclass of HDL, Apo E mediates the uptake of cholesterol, triglycerides, and other lipids. The mature form of Apo E in the human plasma is a single chain glycosylated polypeptide of about 34 kDa containing 299 amino acids.

Three isoforms of Apo E in humans, namely E2, E3, and E4, have been established by isoelectric focusing experiments. They are expressed from a single Apo E genetic locus that gives rise to the three common homozygous phenotypes (E 4/4, E 3/3, E 2/2) and three common heterozygous phenotypes (E 4/3, E 4/2, E 3/2). Studies have shown that E2, with cysteines at positions 112 and 158, has much lower affinity for the LDL receptor. It is associated with prolonged chylomicron-remnant clearance compared to E3 and E4. The E4 isoform, with arginines at 112 and 158, and E3, with cysteine at 112 and arginine at 158, have almost identical affinities for their receptors. The allelic distribution of Apo E correlates well with predisposition to various disease states. The Apo E3 is the most common allele, with homozygotes and heterozygotes accounting for about 60% and 90% of the general population, respectively. Apo E2 homozygotes are considered to be at greater risk for Type III hyperlipoproteinemia and atherosclerosis resulting from the defective binding of Apo E2 to LDL receptors; however, the E2 isoform does exert a protective effect against the development of Alzheimer's disease (AD).

Although Apo E4 has also been linked to atherosclerosis, it has gained more importance in the past decade because of its association with neurodegenerative disorders. The importance of Apo E in the central nervous system became evident due to the correlation of the E4 allele of Apo E with familial and late-onset sporadic AD. Although not all people with Apo E4 develop AD, a significant majority of familial and sporadic cases of AD do have one or two copies of the gene. The presence of the E4 allele increases the risk of AD by accelerating plaque formation and by impairing neuron repair mechanisms. AD patients with the E4 allele are shown to have more amyloid deposits at an earlier age compared to those without the E4 allele. Although the exact mechanism is not known, it is believed that the E4 isoform enhances the deposition and reduces the clearance of Aβ peptide. Apo E4 binds to Aβ peptide at much higher rate than the E3 isoform. Hence, there is a greater Aβ amyloid burden deposited in homozygous E4 AD patients. Apo E is shown to be important for neurite maintenance, and AD patients with E4 show more neuritic deficits than E3 carriers. Apo E4 is also reported to worsen neurological impairment in stroke and multiple sclerosis.

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Apolipoprotein E and Related Products

Product Name	Cat. No.	Size Price	Comments
Apolipoprotein E, Human Plasma, Very Low-Density Lipoprotein	178468	50 µg	A component of VLDL and a subclass of HDL. Present in normal plasma at concentrations of 50 µg/ml. Serves as a ligand for LDL receptors, where it participates in the transport and redistribution of cholesterol and other lipids.
Apolipoprotein E, Isoform E2, Human, Recombinant	178480	50 µg	Isoform bearing cysteine at amino acids 112 and 158. Binds to β-amyloid protein but not to the LDL receptor. Does not compete with human low density lipoprotein for binding to the human Apo B/E (LDL) receptor.

Apolipoprotein E and Related Products, cont.

Product Name	Cat. No. Applications	Size Price	Comments
Apolipoprotein E, Isoform E3, Human, Recombinant	178475 –	50 µg	Isoform bearing cysteine at amino acid 112 and arginine at amino acid 158. Most common form of apolipoprotein E. Binds to β-amyloid protein and to the LDL receptor.
Apolipoprotein E, Isoform E4, Human, Recombinant	178476 –	50 µg	Isoform bearing arginine at amino acids 112 and 158. Binds to β-amyloid protein with higher affinity than Apo E2 and E3. Significantly higher levels of E4 are reported in patients with Alzheimer's disease and atherosclerosis. Promotes early appearance of β-amyloid and neurofibrillary tangles in the elderly.
Anti-Apolipoprotein E, Human (Goat)	178479 ELISA, IB, IP	500 µl	Monospecific for human apolipoprotein E.
Anti-Apolipoprotein E, Human (Mouse)	NE1004 ELISA, FS, IB, IP, PS	100 µl	Detects the E2, E3, and E4 isoforms of apolipoprotein E.

Glycogen Synthase Kinase-3β Inhibitors: Cure for Tauopathies

Glycogen Synthase Kinase-3β (GSK-3β; tau protein kinase I), a highly conserved, ubiquitously expressed serine/threonine protein kinase is involved in the signal transduction cascades of multiple cellular processes. It is negatively regulated by protein kinase B/Akt and by the Wnt signaling pathway. Higher levels of GSK-3β have been shown in pre-tangle and in phosphorylated tau bearing neurons. Overexpression of GSK-3β is a characteristic feature of Alzheimer's disease. GSK-3β accounts for most major phosphorylation sites of fetal and paired helical filament-tau. β-Amyloid peptides are shown to activate GSK-3β, suggesting that activation of GSK-3β is a key mechanism in the pathogenesis of Alzheimer's disease. The development of GSK-3 inhibitors holds considerable promise for reducing tau phosphorylation and the debilitating effects of Alzheimer's disease.

Glycogen Synthase Kinase-Related Products

Product Name	Cat. No. Applications	Size Price	Comments
Aloisine A	128125 –	5 mg	A cell-permeable, potent, selective, reversible, and ATP-competitive inhibitor of Cdks (IC_{50} = 150 nM, 120 nM, 400 nM, and 200 nM for Cdk1/cyclin B, Cdk2/cyclin A, Cdk2/cyclin E, and Cdk5/p25, respectively), glycogen synthase kinase-3 (GSK-3; IC_{50} = 500 nM and 1.5 µM for GSK-3α, GSK-3β, respectively), and c-Jun N-terminal kinase (JNK; IC_{50} ~ 3 - 10 µM).
Aloisine, RP106	128135 –	5 mg	A cell-permeable, potent, selective, ATP-competitive inhibitor of CDK1/cyclin B, CDK5/p35, and GSK-3 (IC_{50} = 700 nM, 1.5 µM, and 920 nM, respectively).
Alsterpaullone	126870 –	1 mg	A potent inhibitor of Cdk1/cyclin B (IC_{50} = 35 nM). Inhibits Tau phosphorylation at sites typically phosphorylated by GSK-3β in Alzheimer's disease. Also inhibits Cdk5/p25-dependent phosphorylation of DARPP-32.
Anti-Glycogen Synthase Kinase, Phospho-Specific ($Ser^{645/649/653/657}$) (Ab-1), Human (Rabbit)	PC457 IB, PS	5 µl 25 µl	Immunogen used was a phosphopeptide corresponding to amino acids 642 - 661 of human glycogen synthase. This sequence contains GSK-3 phosphorylation sites.
Anti-Glycogen Synthase Kinase-3α/β, *Xenopus lacris* (Mouse)	368662 IB	100 µg	Reacts with bovine, canine, Chinese hamster, human, mouse, ovine, porcine, rat, and *Xenopus* glycogen synthase 3α and 3β.
Anti-Glycogen Synthase Kinase 3β, C-Terminal (334-348), Rat (Rabbit)	361528 IB, IP	100 µg	Detects a 47 kDa protein in various tissues of rat and in human A-431 and HeLa cells.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FS:** Frozen Sections; **IB:** Immunoblotting; **IP:** Immunoprecipitation: **PS:** Paraffin Sections

Glycogen Synthase Kinase-Related Products, cont.

Product Name	Cat. No. Applications	Size Price	Comments
Glycogen Synthase Kinase 3β, C-Terminal (334-348) Blocking Peptide, Rat	361529 —	100 µg	A 15-residue (334-348) synthetic peptide based on the rat GSK-3β kinase subdomain XI region. This peptide, coupled to KLH, was used as the immunogen for the production of Anti-GSK-3β (Cat. No. 361528). Suitable for use in immunoabsorption for Western blotting and ELISA.
Glycogen Synthase Kinase 3β-Isozyme, Rabbit Skeletal Muscle, Recombinant, *E. coli*	361526 —	10 KU	Dual specificity kinase. Phosphorylates glycogen synthase. Other substrates include $p90^{rsk}$, Tau, c-Jun, and CREB.
Anti-Glycogen Synthase Kinase-3β, Phospho-Specific (Ser^9) (Ab-1), *Xenopus* (Rabbit)	PC242 DB, ELISA, IB	50 µg	Detects the Ser^9 phosphorylated form of GSK-3β. Does not detect the non-phosphorylated form of GSK-3β.
Glycogen Synthase Kinase-3β Inhibitor I (TDZD-8)	361540 —	5 mg	A thiadiazolidinone (TDZD) analog that acts as a highly selective, non-ATP competitive inhibitor of GSK-3β (IC_{50} = 2 µM). Binds to the active site of GSK-3β. Does not significantly affect the activities of Cdk-1/cyclin B, CK-II, PKA, and PKC (IC_{50} > 100 µM).
Glycogen Synthase Kinase-3 Inhibitor II [2-Thio(3-iodobenzyl)-5-(1-pyridyl)-(1,3,4)-oxadiozole]	361541 —	5 mg	A 2-thio-[1,3,4]-oxadiazole-pyridyl derivative that acts as a potent inhibitor of GSK-3β (IC_{50} = 390 nM).
Glycogen Synthase Kinase-3β Inhibitor III (2,4-Dibenzyl-5-oxothiadiazolidine-3-thione)	361542 —	1 mg	An oxothiadiazolidine-3-thione analog that acts as a non-ATP competitive inhibitor of GSK-3β (IC_{50} = 10 µM).
Glycogen Synthase Kinase-3β Peptide Inhibitor (H-KEAPPAPPQSpP-NH_2)	361545 —	1 mg	A phosphorylated peptide that acts as a substrate-specific, competitive inhibitor of GSK-3β (IC_{50} of 150 µM).
Glycogen Synthase Kinase-3β Peptide Inhibitor, Cell-Permeable (Myr-N-GKEAPPAPPQSpP-NH_2)	361546 —	1 mg	A cell-permeable myristoylated form of GSK-3β Peptide Inhibitor (Cat. No. 361545) with a glycine spacer. Acts as a selective, substrate-specific, competitive inhibitor of GSK-3β (IC_{50} = 40 µM). Displays *in vivo* stability.
Glycogen Synthase Kinase-3β Substrate (H-SPHRSTPESRAAV-OH)	361530 —	1 mg	A part of the hydrophilic loop domain of presenilin 1 that is selectively recognized by GSK-3β. The sequence is not recognized by p38α, p38β, PKC, or CK II. Undergoes phosphorylation at the Ser^{353} and Ser^{357} sites.
Glycogen Synthase Kinase-3β Substrate, Negative Control (H-GPHRATPEARAAV-OH)	361531 —	1 mg	A negative control peptide substrate for GSK-3β substrate (Cat. No. 361530) wherein the Ser^{353} and Ser^{357} are replaced with Ala residues.
Indirubin-3′-monoxime	402085 —	1 mg	A potent inhibitor of GSK-3β (IC_{50} = 22 nM). Also inhibits Cdk1 and Cdk5 (IC_{50} = 180 and 100 nM, respectively).
Indirubin-3′-monoxime, 5-Iodo-	402086 —	1 mg	A highly potent inhibitor of GSK-3β (IC_{50} = 9 nM). Also inhibits Cdk1 (IC_{50} = 25 nM) and Cdk5 (IC_{50} = 20 nM). Binds to GSK-3β's ATP-binding pocket.
Indirubin-3′-monoxime-5-sulphonic Acid	402088 —	1 mg	A potent inhibitor of Cdk1 (IC_{50} = 5 nM), Cdk5 (IC_{50} = 7 nM), and GSK-3β (IC_{50} = 80 nM).
Kenpaullone	422000 —	1 mg	A inhibitor of GSK-3β (IC_{50} = 230 nM), Cdk1 (IC_{50} = 400 nM), and Cdk2 (IC_{50} = 680 nM).

Key: DB: Dot Blot; **ELISA:** Enzyme-Linked Immunosorbent Assay; **IB:** Immunoblotting

Cholinesterase Inhibitors in the Treatment of Alzheimer's Disease

Progressive loss of cholinergic neurons in Alzheimer's disease (AD) patients results in severe memory loss and impairment of cognitive function. Acetylcholinesterase (AChE) is a tetrameric protein that catalyzes the hydrolysis of acetylcholine. The active site of AChE includes a serine hydroxyl group that is rendered more nucleophilic through the proton-acceptor action of a nearby histidine residue. The serine residue exerts a nucleophilic attack on the carbonyl carbon of acetylcholine. AChE inhibitors may act by either competitively blocking hydrolysis without reacting with the enzyme or by acetylating the serine hydroxyl group to form a carbamyl ester, which is more stable than acetate and is less likely to abandon the active site of the enzyme.

One strategy for the treatment of Alzheimer's patients has been to use AChE inhibitors to increase the levels of acetylcholine in the synapse, thereby enhancing cholinergic activity in the affected regions of the brain. AChE inhibitors, which increase the availability of acetylcholine in central synapses, have become the main approach to symptomatic treatment. Clinical studies have indeed shown that patients with mild to moderate AD benefit from treatment with certain AChE inhibitors and muscarinic agonists. These agents do not reverse the progression of the disease, but they do contribute to modest improvements in memory, thinking, and reasoning skills in AD patients. Drugs that are under development may also target butyryl-cholinesterase for additional benefits.

References:

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AChE Inhibitors

Product Name	Cat. No.	Size Price	Comments
Berberine, Hemisulfate	200400	1 g	A reversible and competitive inhibitor of acetylcholinesterase (AChE) with respect to the substrate. Induces apoptosis in promyelocytic leukemia HL-60 cells. Modulates the expression and function of P-glycoprotein 170 (multi-drug resistance transporter) in hepatoma cells. An inhibitor of platelet aggregation induced by ADP, arachidonic acid, and collagen in rats.
Diisopropylfluorophosphate (DFP)	30967	1 g	An organophosphate that acts as a potent irreversible inhibitor of AChE that can cross the blood-brain barrier. Forms a phosphate ester with AChE that cannot be hydrolyzed under physiological conditions.
Galanthamine, Hydrobromide	345670	20 mg	A competitive and reversible inhibitor of AChE Antimyasthenic agent. Can partially reverse the effects of scopolamine-induced amnesia in rats. Reported to improve learning and short-term memory in animal models.
(±)-Huperzine A	385885	1 mg	Synthetic, optically inactive, enantiomeric mixture. Reportedly used as a memory enhancing agent; may act as a cholinomimetic. Inhibits brain AChE activity.
(–)-Huperzine A, *Huperzia serrata*	385886	250 µg	Inhibits AChE (K_i = 8 nM) in a mixed linear competitive manner. More potent enantiomer with three-fold higher activity compared to the racemic mixture (Cat. No. 385885).
Neostigmine Bromide	480200	100 mg	Reversible inhibitor of AChE (IC_{50} = 11.3 nM for electric eel AChE). An inhibitor of the electrogenic sodium pump.
TMTFA	613900	5 mg	A highly potent, reversible, time-dependent, transition state analog inhibitor of AChE (AChE; K_i = 1.3 fM and 15 fM for inhibition of *Electrophorus electricus* and *Torpedo californica* AChE, respectively). Exhibits about 10^{10}-fold tighter binding to AChE than acetylcholine.

The COX Connection in Alzheimer's and Other Neurodegenerative Diseases

For over 30 years, non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit the activity of cyclooxygenase (COX-1 and COX-2) have been the primary drugs used in the treatment of various rheumatological disorders, inflammation, and chronic pain. More recently, there has been an upsurge of interest in COX-2 inhibitors as possible candidates for the treatment of Alzheimer's disease (AD). This is due to the fact that researchers have begun to think about "inflammation as a factor" in the development and/or progression of Alzheimer's disease. Although scientists are not unanimous in their opinion whether inflammation is a cause or an effect of AD, most believe that it contributes to the neuronal damage and that reducing inflammation may help slow or prevent the progression of AD. Several epidemiological studies have shown that groups of people who are on NSAIDs for such conditions as rheumatoid arthritis have a reduced probability of developing AD. It is reported that NSAIDs inhibit human Aβ aggregation *in vitro* and reverse the β-sheet conformation of preformed fibrils. More recently, higher levels of COX-2 have been reported in dopaminergic neurons of Parkinson's disease subjects, which has renewed interest in the application of COX inhibitors in preventing or delaying neurodegenerative disorders.

Cyclooxygenase Inhibitors

Product Name	Cat. No.	Size Price	Comments
COX-2 Inhibitor I (LM-1685)	236011	5 mg	A potent and selective inhibitor of COX-2 (IC_{50} = 650 nM) from human monocyte. Displays only very weak activity against COX-1 from human platelets (IC_{50} > 10 µM) and in whole blood (IC_{50} > 100 µM).
COX-2 Inhibitor, PTPBS	236010	5 mg	A highly selective and potent inhibitor of COX-2 (IC_{50} = 32 nM for COX-2 vs. 55.1 µM for COX-1). Does not exhibit gastric toxicity in a fasting rat model at doses as high as 200 mg/kg.
Curcumin, *Curcuma longa* L.	239802	100 mg	An anti-inflammatory, antitumor agent that inhibits COX (IC_{50} = 52 µM) and 5-lipoxygenase (IC_{50} = 8 µM) activities.
Cyclooxygenase Inhibitor Set	239783	1 set	Contains 100 mg of Meloxicam (Cat. No. 444800), and 5 mg each of NS-398 (Cat. No. 349254), SC-560 (Cat. No. 565610), and Sulindac Sulfide (Cat. No. 574102). Supplied with an informational insert.
Diclofenac, 4′-Hydroxy-	287845	100 µg	A metabolite of Diclofenac (Cat. No. 287840). Formed through oxidation of diclofenac by cytochrome P450 2C9. Suppresses prostaglandin E_2 (PGE_2) formation by specifically blocking COX-2 activity (IC_{50} = 16.9 nM).
Diclofenac Sodium	287840	1 g	Strongly inhibits insoluble transthyretin (TTR) amyloid fibril formation. A potent inhibitor of COX-1 (IC_{50} = 76 nM) and COX-2 (IC_{50} = 26 nM).
DuP-697	317500	5 mg	A potent, irreversible, and time-dependent COX-2 inhibitor. Exhibits over 50-fold greater inhibitory potency against human and murine recombinant COX-2 (IC_{50} = 80 nM and 40 nM at 5 and 10 minutes, respectively) than COX-1 (IC_{50} = 9 µM).
Ebselen	324483	5 mg	A neuroprotective antioxidant that acts as a non-selective inhibitor of the cyclooxygenases. An excellent scavenger of peroxynitrite. Glutathione peroxidase mimetic.
Flufenamic Acid	343075	1 g	A potent inhibitor of NFκB-mediated COX-2 expression. Causes mitochondrial uncoupling via a protonophoric mechanism. Potently inhibits human transthyretin (TTR) amyloid fibril formation.
Flurbiprofen	344079	100 mg	A non-steroidal anti-inflammatory agent that acts as a potent cyclooxygenase inhibitor (IC_{50} = 5 nM for LPS-induced COX in human peripheral blood cells). Reduces Aβ loads and Congo Red staining in APP+PS1 transgenic mice.

Cyclooxygenase Inhibitors, cont.

Product Name	Cat. No.	Size Price	Comments
(±)-Ibuprofen	401003	1 g	A competitive, and non-selective COX inhibitor (IC_{50} = 4.85 µM for COX-1 and 223 µM for COX-2). Decreases the total Aβ secretion ($Aβ_{40\ and\ 42}$) in human neuronal cells and offers neuroprotection against glutamate-, nitric oxide-, and superoxide-induced damage.
(S)-(+)-Ibuprofen	401004	250 mg	A competitive, reversible, and non-selective COX inhibitor (ID_{50} = 8.9 µM for COX-1 and 7.2 µM for COX-2). Reduces the levels of amyloidogenic $Aβ_{42}$ in WT-APP PS1-M146L CHO cells (50% reduction in $Aβ_{42}/Aβ_{40}$ quotient at ~ 200 - 300 µM) and in Tg2576 transgenic mice. Does not affect amyloid precursor protein or Notch processing.
Indomethacin	405268	10 g	A non-steroidal anti-inflammatory, anti-pyretic agent. Non-selective COX inhibitor (IC_{50} = 740 nM for COX-1 and 970 nM for COX-2). Reported to reduce $Aβ_{42}$ load independently of COX inhibition.
Indomethacin Amide, N-Octyl-	405270	5 mg	An N-octylamide derivative of Indomethacin (Cat. No. 405268) that acts as a potent and selective COX-2 inhibitor (IC_{50}= 40 nM) compared to COX-1 (IC_{50} = 66 µM).
Indomethacin Ester, *n*-Heptyl-	405269	5 mg	A heptyl ester derivative of Indomethacin (Cat. No. 405268) that acts as a potent and selective COX-2 inhibitor (IC_{50} = 40 nM) compared to COX-1 (IC_{50} > 66 µM).
Indomethacin Ester, 4-Methoxyphenyl-	405271	5 mg	A 4-methoxyphenyl ester derivative of Indomethacin (Cat. No. 405268) that acts as a potent and selective COX-2 inhibitor (IC_{50} = 40 nM) compared to COX-1 (IC_{50} > 66 µM).
Kaempferol	420345	25 mg	A phytoestrogen that offers protection against $Aβ_{25-35}$-induced cell death in neonatal cortical neurons. Blocks Aβ-induced activation of caspase-2, -3, -8, and -9. Acts as an inhibitor of COX-1 (IC_{50} = 180 µM) and COX-2 (IC_{50} ~15 µM).
Meloxicam	444800	100 mg	Preferentially inhibits COX-2 (IC_{50} = 4.7 µM) relative to COX-1 (IC_{50} = 36.6 µM).
Niflumic Acid	481987	1 g	A selective inhibitor of COX-2 (K_i = 20 nM for sheep placental COX-2).
NS-398	349254	5 mg	Selective inhibitor of COX-2 (IC_{50} = 3.8 µM for sheep placental COX-2). Reduces neuronal damage following a stroke.
Resveratrol	554325	25 mg	A phenolic product with antifungal, antitumor, and antioxidative properties. A specific inhibitor of COX-1 (ED_{50} = 15 µM). Also inhibits the hydroxyperoxidase activity of COX-1 (ED_{50} = 3.7 µM).
SC-236	565605	5 mg	A highly selective and potent inhibitor of COX-2 (IC_{50} = 10 nM for COX-2 vs. IC_{50} = 17.8 µM for COX-1). Exhibits longer half-life and reduced gastric toxicity in fasting rat model.
SC-560	565610	5 mg	A highly potent and selective inhibitor of COX-1 (IC_{50} = 9 nM). Inhibits COX-2 only at higher concentrations (IC_{50} = 6.3 µM).
SC-58125	565620	5 mg	A potent and selective inhibitor of COX-2 (IC_{50} = 50 nM). Weakly but rapidly inhibits COX-1 (IC_{50} > 10 µM) in a competitive and reversible manner. Also exhibits a similar profile for human recombinant COX enzymes.
Sulindac	574100	1 g	A prodrug that is metabolized to a pharmacologically active sulfide derivative that potently inhibits cyclooxygenase activity. Inhibits chemical carcinogenesis in rodent models and causes regression of adenomas by an apoptotic mechanism.
Sulindac Sulfide	574102	5 mg	A selective inhibitor of COX-1 (ID_{50} = 500 nM) versus COX-2 (ID_{50} = 14 µM). Reduces $Aβ_{42}$ load independently of COX inhibition.
Sulindac Sulfone	574105	5 mg	A sulfone metabolite of Sulindac (Cat. No. 574100) that has anti-cancer properties but lacks cyclooxygenase (COX) inhibitory activity. Also inhibits cell growth and induces apoptosis.

Alzheimer's Disease: Misguided Slicing of Amyloid Precursor Protein by Secretases

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Alzheimer's disease (AD) is characterized by a progressive deposition of the 4 kDa β-amyloid peptide (Aβ) in senile plaques and accumulation of Tau protein as neurofibrillary tangles. In normal healthy individuals, Aβ peptides are present only in small quantities as soluble monomers that circulate in cerebrospinal fluid and blood. However, in AD patients, their levels increase significantly and they begin to accumulate as insoluble, fibrillar plaques. Aβ's are 40 to 43 amino acid peptides that originate from the proteolytic cleavage of the amyloid precursor protein (APP). APP is reported to occur in three common isoforms, APP695, APP751, and APP770. APP695 is expressed exclusively in neurons, whereas APP751 and APP770 are present in both neural and non-neural cells. The primary structure of APP contains a small signal sequence, a large extramembranous N-terminal region, a single transmembrane domain, and a cytoplasmic C-terminal tail. Processing of APP *in vivo* occurs by two major pathways. Cleavage of APP at the N-terminus of the Aβ region by β-secretase and at the C-terminus by γ-secretase represents the amyloidogenic pathway for processing of APP. The β-secretase cleaves APP between residues Met^{671} and Asp^{672} and yields sAPPs and the C99 fragment. The β-secretase has also been identified as an aspartyl protease (BACE or Asp-2) of unusual nature. It has a C-terminal transmembrane domain and two active site motifs located in the luminal domain. Newly synthesized BACE contains a propeptide domain, which is cleaved at residue E46 to produce the mature enzyme. The active site of BACE and the β-secretase cleavage site of APP are in precise topological orientation for endoproteinases. Succeeding the β-secretase cleavage, a second cleavage occurs at the C-terminus of Aβ peptide that releases Aβ from C99. This cleavage occurs in the vicinity of residue 712 of the C-terminus. The γ-secretase can cleave the C-terminal region at either Val^{711} or Ile^{713} to produce the shorter Aβ peptide ($Aβ_{1-40}$) or the longer Aβ peptide ($Aβ_{1-42}$). The predominant form of Aβ found in the cerebrospinal fluid is the shorter $Aβ_{1–40}$ peptide. Despite its lower rate of synthesis, $Aβ_{1–42}$ is the peptide that is initially deposited within the extracellular plaques of AD patients. In addition, $Aβ_{1–42}$ is shown to aggregate at a much lower concentration than the $Aβ_{1–40}$ form.

APP can also be processed by α-secretase (TACE), which cleaves within the Aβ domain between Lys^{687} and Leu^{688} and produces a large soluble α-APP domain and the C-terminal fragment containing P3 and C83. The latter can then be cleaved by γ-secretase at residue 711 or 713 to release P3 fragment. This pathway does not yield Aβ peptide. Hence, shunting APP towards the α-secretase pathway may have a beneficial effect in lowering Aβ peptide levels. It is reported that α-secretase shares many of its properties with the secretase that cleaves angiotensin-converting enzyme and is believed to be a zinc metalloproteinase of the ADAMs family. Muscarinic agonists (M1 and M3) and some PKC activators are reported to enhance α-secretase activity and are under consideration for their therapeutic value as AD treatment tools.

Presenilin (PS) and nicastrin have also been reported to play an important role in βAPP processing. PS1 and PS2 are polytopic transmembrane proteins that share extensive amino acid sequence identity. They are functional components of separate high molecular weight complexes in the endoplasmic reticulum (ER) and Golgi apparatus and are essential for the proteolytic cleavage of several proteins, including βAPP and Notch. γ-secretase cleavage of βAPP and production of $Aβ_{1-42}$ is reported to increase as a result of mutations in PS1 and PS2. Some researchers have suggested that PS may have some catalytic activity of their own and may even be γ-secretases, while others argue that this activity requires interactions between the presenilins and other proteins. PS1 and PS2 also complex with nicastrin, a transmembrane glycoprotein that is important in PS-mediated βAPP and Notch processing. Nicastrin binds both full-length βAPP and γ-secretase substrates (C99-

and C83-βAPP fragments), and modulates the activity of γ-secretase. Mutations in the conserved domain of nicastrin (312-369 in the hydrophilic N-terminus) increase βAPP cleavage by γ-secretase; however, binding of nicastrin to C99-/ C83- is not significantly altered.

In mammalian cells, endogenous as well as over-expressed nicastrin undergoes post-translational *N*-glycosylation during trafficking from the endoplasmic reticulum to the Golgi apparatus. PS1 is shown to interact preferentially with mature glycosylated nicastrin. Studies using an RNA interference approach has shown that down-regulation of the nicastrin level leads to accumulation of the C-terminal fragments of the βAPP, destabilizes PS, and leads to reduction in Aß production. It is suggested that nicastrin and PS regulate each other and determine γ-secretase activity via formation of a complex. Interestingly, inhibition of nicastrin expression is shown to reduce γ-secretase activity and PS1 complex formation. This indicates that perhaps nicastrin is a limiting factor for the assembly of PS complex. In addition to nicastrin, recent genetic studies have identified a number of additional genes encoding Aph-1α, Aph-1β, and Pen-2 proteins, as part of the γ-secretase complex with PS1. Over-expression of these proteins can also increase the levels of Aβ, which indicates their limiting nature for γ-secretase activity.

Most cases of familial AD (FAD) are reported to result from mutations in one of the three genes, APP, PS1 and PS2. The mutation in the APP gene, located on chromosome 21, accounts for about 2% of all cases of FAD and approximately 5 - 20% of early-onset FAD. Over 40 different mutations have been reported in PS1, which account for about 30 to 50% of all presenile FAD. The PS2 gene mutations are rather rare and account for less than 2% of all early-onset FAD. Mutations in both PS1 and PS2 are associated with an increased production of the $A\beta_{1-42}$ peptide.

The characterization of the APP secretases during the past few years has provided significant advancement in therapeutic strategies that may lead to limiting the build up of Aβ peptide in the brain and eliminating or delaying the pathological effects of AD. Recent characterization of secretases has uncovered several common features, particularly their sensitivity to certain metalloprotease inhibitors and the upregulation of their activity by phorbol esters. Presenilins and γ-secretases are considered to be the most promising molecular targets for developing therapeutic agents that may minimize the debilitating effects of AD. Major focuses in AD research are identifying more genetic and environmental factors responsible for Aβ build-up in nerve cells.

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Presenilin-Related Antibodies

Product Name	Cat. No. / Applications	Size / Price	Comments
Anti-Nicastrin, C-Terminal (689-709), Human (Rabbit)	**481905** IB, IP, RIA	100 µl	Recognizes both the immature and mature forms of nicastrin. Reacts with human and mouse.
Anti-Nicastrin, N-Terminal (62-93), Human (Rabbit)	**481906** IB, IF, IP	100 µl	Recognizes both the immature and mature forms of nicastrin. Reacts with human and mouse.
Anti-PEN-2, N-Terminal, Human (Rabbit)	**NE1008** IB,IP	50 µl	Detects the ~10 kDa integral membrane protein PEN-2. PEN-2 is reported to physically interact with presenillin 1 and be necessary for γ-secretase activity.
Anti-Presenilin 1 (31-46) (Ab-1), Mouse (Rabbit)	**PC244** IB, IF	10 µg 50 µg	Detects a 98 kDa band by Western blot which is thought to be the PS1 dimer. A 30 kDa endoproteolytic fragment is also detected. Reacts with mouse and rat.
Anti-Presenilin 1 (303-316) (Ab-2), Mouse (Rabbit)	**PC267** IB, IF	10 µg 50 µg	Detects a 98 kDa band by Western blot which is thought to be the PS1 dimer. A 21 kDa endoproteolytic fragment is also detected. Reacts with mouse and rat.
Anti-Presenilin 1 (14-33) (Ab-3), Human (Goat)	**PC361** ELISA, FS, PS	100 µl	Recognizes human presenilin 1.
Anti-Presenilin 1, Loop Domain (263-407), Human (Rabbit)	**529592** IB, IF, IP	100 µl	Polyclonal that recognizes the presenilin 1 holoprotein and its C-terminal fragments.
Anti-Presenilin 1, N-Terminal (14-33), Human (Goat)	**529586** ELISA, IH	100 µl	Specific for residues 14 - 33 on the N-terminus of presenilin-1.
Anti-Presenilin 1, N-Terminal (1-65), Human (Rabbit)	**529591** IB, IF, IP	100 µl	Recognizes the human and mouse presenilin 1 holoprotein and its N-terminal fragments.
Anti-Presenilin 2, N-Terminal (31-47), Human (Goat)	**529587** ELISA, FS	100 µl	Recognizes 50 kDa human presenilin 2. Labels both normal and Alzheimer's disease brain neurons.
Anti-Presenilin 2 (7-24) (Ab-1), Human (Rabbit)	**PC305** FS, IB **PC305T** FS, IB	10 µg 25 µg 5 µg	Recognizes an N-terminal proteolytic fragment of ~28 kDa. Additional bands are also observed which likely correspond to modified, aggregated, or intact PS2 depending on extraction conditions. Reacts with human, mouse, and rat.
Anti-Presenilin 2 (324-335) (Ab-2), Human (Rabbit)	**PC235** FS, IB **PC235T** FS, IB	25 µg 5 µg	Recognizes a C-terminal proteolytic fragment at 20 kDa in Western blot using mouse or rat brain tissue extract, as well as in HeLa cell extract. Additional bands are also observed which likely correspond to aggregated, modified, or intact PS2 depending on extraction conditions.
Anti-Presenilin 2, Loop Domain (269-394) Human (Rabbit)	**529594** IB, IF, IP	100 µl	Recognizes the human and mouse presenilin 2 holoprotein and its C-terminal fragments.
Anti-Presenilin 2, N-Terminal (1-75), Human (Rabbit)	**529593** IB, IF, IP	100 µl	Recognizes the human and mouse presenilin 2 holoprotein and its N-terminal fragments.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FS:** Frozen Sections; **IB:** Immunoblotting; **FFS:** Free-Floating Sections; **IF:** Immunofluorescence; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections; **RIA:** Radioimmunoassay

Presenilin 1 Antisense Oligonucleotides

Product Name	Cat. No.	Size / Price	Comments
Presenilin 1 Antisense Oligonucleotide, Sodium Salt	**529595**	50 nmol	An 18-meric phosphorothioate oligonucleotide, complimentary to sequences flanking the initiation codon of the mouse PS1 gene, that is shown to significantly reduce NMDA-induced cell death in primary cortical neurons (~50% inhibition at 3 mM) and PS1 mRNA levels. Displays no effect on cell viability.

Presenilin 1 Antisense Oligonucleotides, cont.

Product Name	Cat. No.	Size Price	Comments
Presenilin 1 Antisense Oligonucleotide, Negative Control, Sodium Salt	529596	50 nmol	The sense sequence of the PS1 AS (Cat. No. 529595) that serves as a negative control for PS1 AS.
Presenilin 1 Antisense Oligonucleotide, Fluorescein-Labeled, Sodium Salt	529597	10 nmol	PS1 AS (Cat. No. 529595) labeled at the 5' terminal with the fluorescent tag, 6-FAM [(6-fluorescein-6-carboxamido) hexanoate]. Useful for monitoring and confirming cellular uptake of the PS1 AS.

Secretase Inhibitors

Product Name	Cat. No.	Size Price	Comments
APP β-Secretase Inhibitor (H-KTEEISEVN-Stat-VAEF-OH)	171601	500 µg	A potent inhibitor of the amyloid precursor protein (APP) β-secretase (IC_{50} = 30 nM).
MG-132	474790	1 mg 5 mg	A potent, reversible, and cell-permeable proteasome inhibitor (K_i = 4 nM). Reduces the degradation of ubiquitin-conjugated proteins in mammalian cells and permeable strains of yeast by the 26S complex without affecting its ATPase or isopeptidase activities. Blocks the maturation of APP Swedish mutant (APPSw) preventing cleavage by β-secretase. Inhibits NF-κB activation (IC_{50} = 3 µm).
OM99-2	496000	250 µg	A peptidomimetic, highly potent, tight-binding transition-state analog inhibitor of β-secretase (K_i = 1.6 nM, recombinant memapsin-2; K_i = 9.58 nM, recombinant pro-memapsin 2). Designed from the template of the β-secretase site of Swedish β-amyloid precursor protein (APP) with Asp to Ala replacement.
Pepstatin A	516482	5 mg 25 mg 100 mg 250 mg	A reversible, tightly binding inhibitor of aspartic proteases. Inhibits cathepsin D, pepsin, and renin. Reported to block apoptosis in PC12 cell lines. Inhibits γ-secretase activity.
Pepstatin A Methyl Ester	516485	1 mg 5 mg	A cell-permeable methyl ester derivative of Pepstatin A (Cat. No. 516482) that acts as a potent, non-competitive, transition-state analog inhibitor of γ-secretase (K_{is} = 150 nM, K_{ii} = 320 nM for human γ-secretase at 20°C); K_{is} is the inhibition constant for inhibitor binding to the free enzyme and K_{ii} is the inhibition constant for inhibitor binding to the Enzyme-Substrate complex).
β-Secretase Inhibitor II (Z-VLL-CHO)	565749	1 mg 5 mg	A potent, cell-permeable, and reversible inhibitor of β-secretase. Corresponds to the β-secretase cleavage site (VNL-DA) of the Swedish mutant Amyloid Precursor Protein (APP). Inhibits the formation of both $Aβ_{total}$(IC_{50} = 700 nM) and $Aβ_{1-42}$ (IC_{50} = 2.5 µM) in Chinese hamster ovary (CHO) cells transfected with wild-type APP751.
β-Secretase Inhibitor III	565780	500 µg	A substrate analog inhibitor of β-secretase (BACE) that completely blocks the proteolytic activity (at 5 mM) in solubilized membrane fractions from BACE transfected MDCK cells.
γ-Secretase Inhibitor I (Z-LLNle-CHO)	565750	1 mg	Inhibits γ-secretase activity.

Product Name	Cat. No.	Size Price	Comments
γ-Secretase Inhibitor II	565755	1 mg	A reversible and selective peptidomimetic inhibitor of γ-secretase (IC_{50} = 13 µM for total inhibition of Aβ). Displays only weak inhibitory activity against calpain II (IC_{50} = 100 µM in a purified enzyme assay).
γ-Secretase Inhibitor III (Z-LL-CHO)	565760	1 mg 5 mg	A cell-permeable, specific, and reversible inhibitor of γ-secretase that reduces the formation of both $Aβ_{total}$ (IC_{50} ~ 35 µM) and $Aβ_{1-42}$ in Chinese hamster ovary (CHO) cultures stably transfected with amyloid precursor protein-751. Reported to be nontoxic in nature.
γ-Secretase Inhibitor IV (2-Naphthoyl-VF-CHO)	565761	1 mg	A cell-permeable, reversible inhibitor of γ-secretase. Inhibits the release of $Aβ_{x-40}$ (ED_{50} = 2.6 µM) and $Aβ_{x-42}$ (ED_{50} = 2.7 µM) in HEK293 cells stably transfected with the APP Swedish mutants.
γ-Secretase Inhibitor V (Z-LF-CHO)	565762	1 mg	A cell-permeable, reversible inhibitor of γ-secretase. Reported to inhibit the release of $Aβ_{x-40}$ (ED_{50} = 5.0 µM) in HEK293 cells stably transfected with the APP Swedish mutants.
γ-Secretase Inhibitor VI [1-(S)-*endo*-N-(1,3,3)-Trimethylbicyclo[2.2.1]hept-2yl-4-fluorophenyl Sulfonamide]	565763	5 mg	A cell-permeable inhibitor of $Aβ_{42}$ production (IC_{50} = 1.8 µM). Treatment of HEK293 cells with this inhibitor results in an increase in β-secretase-cleaved APP fragments and secreted $APP_sα$.
γ-Secretase Inhibitor VII (MOC-LL-CHO)	565768	1 mg	A cell-permeable, reversible inhibitor of Aβ and p3 secretion ($Aβ_{40}$ IC_{50} = 2.3 µM; $Aβ_{42}$ IC_{50} = 3 µM). Reported to be more potent (IC_{50} = 900 nM and 740 nM for $Aβ_{40}$ and $Aβ_{42}$, respectively) in the presence of C99 Inhibitor (10 µM; Cat. No. 205533).
γ-Secretase Inhibitor IX (DAPT)	565770	5 mg	A cell-permeable dipeptide that reduces Aβ production by blocking γ-secretase ($Aβ_{total}$ IC_{50} = 115 nM, $Aβ_{42}$ IC_{50} = 200 nM). Reported to be functionally active in both HEK293 cells and neuronal cultures without affecting the secretion of amyloid-β precursor protein.
γ-Secretase Inhibitor X (L-685,458)	565771	250 µg	A cell-permeable, highly specific and potent inhibitor of γ-secretase ($Aβ_{total}$ IC_{50} = 17 nM, $Aβ_{40}$ IC_{50} = 48 nM, and $Aβ_{42}$ IC_{50} = 67 nM in SH-SY5Y cells overexpressing spβA4CTF). Binds to presenilin and blocks Notch intracellular domain production. Functions as a transition state analog mimic at the catalytic site of an aspartyl protease. Exhibits over 100-fold greater selectivity for γ-secretase than for cathepsin D.
γ-Secretase Inhibitor XI (7-Amido-4-chloro-3-methoxyisocoumarin)	565772	5 mg	An active site-directed, irreversible serine protease inhibitor that acts as a highly selective, potent inhibitor of γ-secretase. Blocks production of both amyloid-$β_{40}$ ($Aβ_{40}$) and $Aβ_{42}$ (IC_{50} < 100 µM) in HEK293 cells expressing wild-type and Swedish mutant β-amyloid precursor protein.
γ-Secretase Inhibitor XII (Z-IL-CHO)	565773	5 mg	A cell-permeable, reversible dipeptide aldehyde that reduces Aβ production by blocking γ-secretase *in vitro* ($Aβ_{40}$ IC_{50} = 7.9 µM; $Aβ_{42}$ IC_{50} = 7.6 µM) and in cultured CHO cells that stably overexpress APP695 ($Aβ_{40}$ IC_{50} = 11.5 µM; $Aβ_{42}$ IC_{50} = 8.3 µM). Also blocks the generation of γ CTF (γ-secretase-generated C-terminal fragment). Does not affect the formation of amyloid-β precursor protein.
γ-Secretase Inhibitor XIII (Z-YIL-CHO)	565774	5 mg	A cell-permeable, reversible inhibitor of γ-secretase. In TPA-stimulated T47-14 cells, it abolishes nuclear localization of ErbB-4 receptor tyrosine kinase by inhibiting the formation of the s80 ErbB-4 fragment.
γ-Secretase Inhibitor XIV [Z-C(t-Bu)-IL-CHO]	565775	5 mg	A cell-permeable, reversible inhibitor of γ-secretase that reduces Aβ production ($Aβ_{40}$ IC_{50} = 190 nM; $Aβ_{42}$ IC_{50} = 780 nM) in solubilized membrane preparations and in cultured APP695 expressing CHO cells ($Aβ_{40}$ IC_{50} = 80 nM; $Aβ_{42}$ IC_{50} = 120 nM).
γ-Secretase Inhibitor XVI (DAPM)	565777	5 mg	A cell-permeable γ-secretase inhibitor with anti-aggregation property (Aβ IC_{50} ~10 nM in 7PA2 cells). Prevents early Aβ oligomerization by selectively blocking the Aβ dimer and trimer formation.

Secretase Inhibitors, cont.

Product Name	Cat. No.	Size Price	Comments
γ-Secretase Inhibitor XVII (WPE-III-31C)	565778	500 µg	A cell-permeable (hydroxyethyl)urea peptidomimetic that acts as a transition-state analog inhibitor of γ-secretase (IC_{50} = 300 nM for Aβ production in whole cells). Binds the presenilin-γ-secretase complex (PS1-NTF, PS1-CTF, Nicastrin, and C83 APP CTF).
γ-Secretase Inhibitor XVIII (Compound E)	565779	250 µg	A cell-permeable peptidyl dihydrobenzodiazepinone derivative that acts as a highly potent, selective, non-transition state and non-competitive inhibitor of γ-secretase (IC_{50} $Aβ_{total}$ = 300 pM in CHO cells overexpressing wild-type βAPP). Binds to the active site of PS1 and PS2.
$γ_{40}$-Secretase Inhibitor I (t-3,5-DMC-IL-CHO)	565765	1 mg	A potent cell-permeable, reversible inhibitor of γ-secretase that preferentially inhibits the secretion of $Aβ_{1-40}$ (> 90%) vs. $Aβ_{1-42}$ (~15%). IC_{50} = ~15 µM for $Aβ_{total}$; ~22 µM for $Aβ_{1-40}$; and > 50 µM for $Aβ_{1-42}$ in CHO cells stably transfected with the cDNA encoding βAPP695. Reported to be about 10-fold more potent than Z-Val-Phe-CHO (MDL 28170; Cat. No. 208722).
$γ_{40}$-Secretase Inhibitor II (BOC-GVV-CHO)	565766	1 mg 5 mg	A cell-permeable substrate-based ($γ_{40}$-site) γ-secretase inhibitor that is reported to preferentially (> 90%) inhibit Aβ cleavage at site 40 vs. 42, in a dose-dependent fashion, in transiently transfected 293T cells over-expressing APP695NL.

Secretase Substrates

Product Name	Cat. No.	Size Price	Comments
Furin Substrate, Fluorogenic	344935	5 mg	A fluorogenic substrate for furin, a mammalian homolog of the yeast Kex2 endoprotease (k_{cat}/K_m ~ 2 x 10^4 $M^{-1}s^{-1}$).
α-Secretase Substrate I, Fluorogenic [MCA-HQKLVFFAK(DNP)-NH_2]	565751	500 µg	A specific fluorescent resonance energy transfer (FRET) substrate for the detection of α-secretase activity. Ex. max.: ~ 325 nm; Em. max.: ~ 393 nm.
α-Secretase Substrate, Control [H-VFFAK(DNP)-NH_2]	565752	500 µg	A control peptide for the α-Secretase Substrate I, Fluorogenic (Cat. No. 565751).
α-Secretase Substrate II, Fluorogenic [Ac-RE(EDANS)-VHHQKLVF-K(DABCYL)-R-OH]	565767	1 mg	An internally quenched fluorogenic peptide substrate containing the α-secretase cleavage site of the β-amyloid precursor protein (APP). Ex. max.: ~340 nm; Em. max.: ~490 nm.
β-Secretase Substrate I, Fluorogenic [MCA-EVKMDAEFK(DNP)-NH_2]	565753	500 µg	A specific fluorescent resonance energy transfer (FRET) substrate for the detection of β-secretase activity. Ex. max.: ~325 nm; Em. max.: ~393 nm.
β-Secretase Substrate, Control [H-DAEFK(DNP)-NH_2]	565754	500 µg	A control for the β-Secretase Substrate I, Fluorogenic (Cat. No. 565753).
β-Secretase Substrate II (H-SEVNLDAEFR-OH)	565756	1 mg	A useful substrate for assaying β-secretase activity. This peptide corresponds to the Swedish KM→NL mutation of the amyloid precursor protein (APP) β-secretase cleavage site. Exhibits a significantly higher rate of hydrolysis than the wild-type substrate (Cat. No. 565757).
β-Secretase Substrate III (H-SEVKMDAEFR-OH)	565757	1 mg	A useful substrate for assaying β-secretase activity. Contains the wild-type APP β-secretase cleavage site.
β-Secretase Substrate IV, Fluorogenic [H-RE(EDANS)EVNLDAEFK-(DABCYL)R-OH]	565758	1 mg	A highly sensitive fluorescence resonance energy transfer (FRET) substrate, based on the EVNLDAEF sequence derived from the β-secretase site of the Swedish mutation of amyloid precursor protein (APP). Hydrolysis of the substrate at Leu-Asp bond by β-secretase results in fluorescence enhancement. Reported k_{cat}/K_m values ($min^{-1}/µM$) at pH 4.5, 37°C for pro-mempasin 2 and its proteolytically activated species Leu^{28p}-mempasin 2 are 0.044 and 0.040, respectively. Ex. max.: ~350 nm; Em. max.: ~490 nm.

Secretase Substrates, cont.

Product Name	Cat. No.	Size Price	Comments
β-Secretase Substrate V, Fluorogenic [MCA-SEVNLDAEFK(DNP)-$CONH_2$]	565759	1 mg	A highly sensitive fluorescence resonance energy transfer (FRET) substrate, based on the EVNLDAEF sequence derived from the β-secretase site of the Swedish mutation of amyloid precursor protein (APP). Hydrolysis of the substrate at Leu-Asp bond by β-secretase results in fluorescence enhancement. Reported k_{cat}/K_m values ($min^{-1}/\mu M$) at pH 4.5, 37°C for pro-mempasin 2 and its proteolytically activated species Leu^{28p}-mempasin 2 and Gly^{45p}-mempasin 2 are 0.056, 0.080, and 0.160, respectively. Ex. max.: ~ 328 nm; Em. max.: ~ 393 nm.
β-Secretase Substrate VI, Fluorogenic [H-K(DABSYL)-SEVNLDAEFRQ(LY)]	565781	500 µg	A highly selective, fluorescence resonance energy transfer (FRET) peptide substrate for β-secretase (BACE); (pH ~ 4.0, k_{cat} = 0.02 sec^{-1}; K_m = 9 µM). Derived from the Swedish mutant APP (amyloid precursor protein) β-cleavage site (-NL↓DA-). Useful for high throughput screening of β-secretase inhibitors.
γ-Secretase Substrate, Fluorogenic [(NMA-GGVVIATVK(DNP)-*DRDRD*R-NH_2]	565764	1 mg	An internally quenched fluorogenic peptide substrate containing the C-terminal β-APP amino acid sequence that is cleaved by γ-secretase. Sensitive and useful for assaying γ-secretase activity. The proteolysis at the $Aβ_{40}$-, $Aβ_{42}$-, and $Aβ_{43}$-generating cleavage sites results in enhanced fluorescence.
β-Secretase Substrate VII, Fluorogenic (Abz-VKMDAE-EDDnp)	565782	1 mg	An internally quenched fluorogenic peptide substrate designed from wild-type β-APP sequence that selectively detects BACE1 (β-secretase 1), BACE2, and cathepsin D. Cleavage occurs between Met-Asp residues and results in fluorescence enhancement. Also useful for screening of inhibitors for BACE1, BACE2, and cathepsin D.
β-Secretase Substrate VIII, Fluorogenic (Abz-VNLDAE-EDDnp)	565783	1 mg	An internally quenched fluorogenic peptide substrate designed from Swedish mutated β-APP sequence that specifically detects the activity of BACE1 (β-secretase 1) and BACE2, but not that of cathepsin-D, ADAM-10, TACE, PS1, or PS2. Cleavage occurs between Leu-Asp residues and results in fluorescence enhancement. Also useful for screening of inhibitors for BACE1 and BACE2.

β-Secretase Kit

Product Name	Cat. No.	Size Price	Comments
β-Secretase Activity Assay Kit, Fluorogenic	565785	1 Kit*	A sensitive fluorogenic assay for the determination of β-secretase (BACE) activity. β-Secretase is a transmembrane aspartyl protease which cleaves membrane-bound amyloid precursor protein.

*1 Kit = 100 assays.

Tau Protein: Hyperphosphorylation and Alzheimer's Disease

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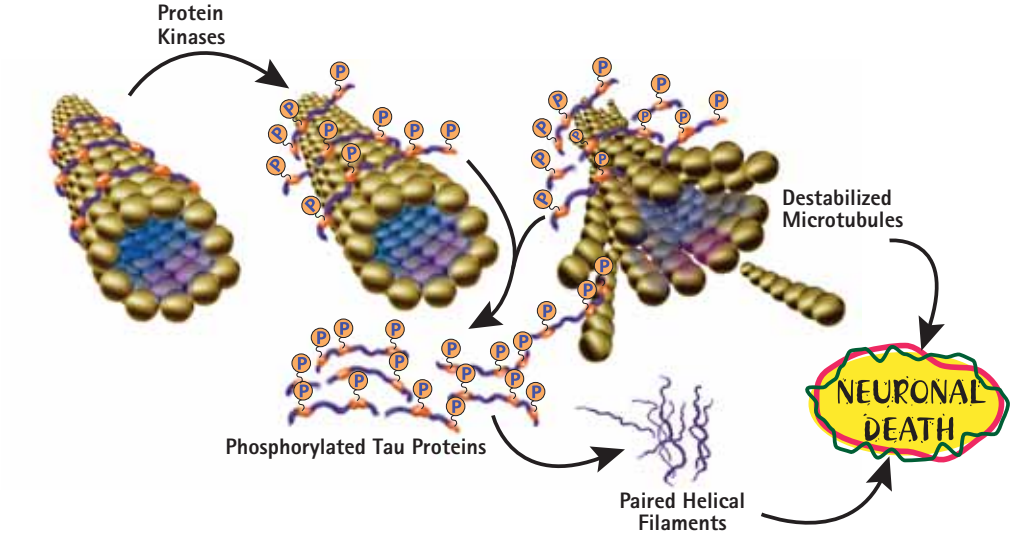


Tau, a highly asymmetric and heat-stable protein, is expressed mainly in the brain, where it regulates the stability and orientation of microtubules in neurons, astrocytes, and oligodendrocytes. Tau is highly enriched in the axon where it is involved in anterograde transport. The major function of Tau is to stabilize microtubules and to bundle microtubules in the axon. Tau protein is reported to be the predominant component of the paired helical filaments (PHFs) and neurofibrillary tangles (NFTs) that are characteristic of pathological brain lesions in Alzheimer's disease.

The dephosphorylation of Tau protein promotes rapid and extensive microtubule polymerization, while the phosphorylation of Tau decreases its ability to promote microtubule assembly. Tau protein, especially in Alzheimer's brains, is phosphorylated at many different sites. *In vitro* studies have shown that Tau is a substrate for a multitude of protein kinases, including CaM kinase II, casein kinase II, protein kinase A (PKA), MAP kinase (ERK2), Cdk5/ p35, and glycogen synthase kinase-3 (GSK-3). It is believed that the phosphorylation of specific sites, rather than the overall extent of phosphorylation, is important in modulating the ability of Tau to bind microtubules and promote microtubule assembly. For example, phosphorylation of Tau by PKA decreases tubulin binding, while phosphorylation by CaM kinase II does not appear to do so. Tau is extensively phosphorylated by ERK2 *in vitro*, and this phosphorylation decreases its affinity for paclitaxel-stabilized microbutules. Phosphorylation of Tau at Ser^{262} and Ser^{356}, within the microtubule-binding regions, by $p110^{mark}$ (MARK) virtually abolishes the ability of Tau to bind microtubules. The role of protein phosphatases in Tau phosphorylation state has also gained attention in recent years. Phosphorylated Tau is shown to be dephosphorylated by protein phosphatase 1 (PP1), protein phosphatase 2A (PP2A), and protein phosphatase 2B (PP2B). Upon dephosphorylation the ability of Tau to bind microtubules and promote microtubule assembly is significantly increased.

In Alzheimer's disease brain, PHFs exist in an insoluble state as a component of the NFTs. *In vitro* studies have shown that the ability of insoluble PHFs to bind microtubules is significantly reduced and can be restored upon dephosphorylation of PHF-Tau. The hyperphosphorylated PHF-Tau is highly resistant to degradation by calpain.

It is believed that the hyperphosphorylation of Tau alone is not sufficient to induce PHF formation *in vivo*. A decrease in redox potential and the resulting oxidizing environment also

plays an important role in the Tau-associated pathology in Alzheimer's disease. Self-association of Tau proteins is reported to be much higher in an oxidizing environment. Tau-Tau dimer formation occurs via intermolecular disulfide linkages. Increased Tau aggregation due to a more oxidizing environment also results in Tau glycation. Proteins susceptible to glycation are usually long-lived and often have high lysine content - Tau has 44 lysine residues. PHF-Tau from the temporal cortex of Alzheimer's disease patients is shown to be glycated, while normal control Tau, or soluble Tau from Alzheimer's disease brains, is not glycated. PHF-Tau, which is both glycated and hyperphosphorylated, exhibits a greater reduction in microtubule binding compared to soluble Tau from Alzheimer's brain that is hyperphosphorylated, but not glycated.

Tau Antibodies

Product Name	Cat. No.	Size	Comments
		Price	
Anti-Tau, Bovine (Mouse) (Clone: Tau-5)	577801 FS, IB, IP, PS	100 µg	Recognizes Tau proteins ranging from 45 to 68 kDa in human, mouse, rat, and sheep. Cross-reacts with both native and phosphorylated Tau protein.
Anti-Tau (Ab-1), Bovine (Mouse) (Clone: Tau-2)	NB16 FS, IB, IF	100 µg	Non-phosphorylated splice variants of Tau (45 - 68 kDa). Reacts with bovine, chicken, feline, human, and monkey.
Anti-Tau, Phospho-Specific (Ser^{199}, Ser^{202}), Human (Rabbit)	577802 IB	10 T	Recognizes Tau proteins phosphorylated at Ser^{199} and Ser^{202}. Reacts with human, mouse, and rat.
Anti-Tau, Phospho-Specific (Thr^{181}), Human (Rabbit)	577804 IB	10 T	Reacts with human Tau phosphorylated at Thr^{181}.
Anti-Tau, Phospho-Specific (Thr^{181}), Mouse (Rabbit)	577806 IB	10 T	Reacts with mouse and rat Tau phosphorylated at Thr^{181}.
Anti-Tau, Phospho-Specific (Ser^{199}), Human (Rabbit)	577807 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Ser^{199} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{202}), Human (Rabbit)	577808 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Ser^{202} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Thr^{205}), Human (Rabbit)	577809 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Thr^{205} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Thr^{212}), Human (Rabbit)	577810 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Thr^{212} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{214}), Human (Rabbit)	577811 IB	10 T	Reacts with Tau protein phosphorylated at Ser^{214} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Thr^{217}), Human (Rabbit)	577812 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Thr^{217} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Thr^{231}), Human (Rabbit)	577813 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Thr^{231} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{262}), Human (Rabbit)	577814 IB	10 T	Reacts with Tau protein phosphorylated at Ser^{262} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{396}), Human (Rabbit)	577815 IB	10 T	Reacts with Tau protein phosphorylated at Ser^{396} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{400}), Human (Rabbit)	NE1006 IB	10 T	Detects Tau protein phosphorylated on Ser^{400}, which can be phosphorylated by GSK-3β.
Anti-Tau, Phospho-Specific (Ser^{404}), Human (Rabbit)	NE1007 IB	10 T	Detects Tau protein phosphorylated on Ser^{404} which can be phosphorylated by GSK-3β, and Cdk5.
Anti-Tau, Phospho-Specific (Ser^{409}), Human (Rabbit)	577816 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Ser^{409} in human, mouse, and rat.
Anti-Tau, Phospho-Specific (Ser^{422}), Human (Rabbit)	577817 ELISA, IB, IH	10 T	Reacts with Tau protein phosphorylated at Ser^{422} in human, mouse, and rat.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FS:** Frozen Sections; **IB:** Immunoblotting; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections; 1 T = 1 Test

Tau-Related Research Tools

Product Name	Cat. No.	Size Price	Comments
Glycogen Synthase Kinase 3β-Isozyme, Rabbit Skeletal Muscle, Recombinant, *E. coli*	361526	10 KU	Dual specificity protein kinase that phosphorylates glycogen synthase. Other substrates include $p90^{rsk}$, Tau, c-Jun, and CREB.
Indirubin-3′-monoxime, 5-Iodo-	402086	1 mg	A highly potent inhibitor of glycogen synthase kinase-3β (GSK-3β; IC_{50} = 9 nM). Also inhibits Cdk1 (IC_{50} = 25 nM) and Cdk5 (IC_{50} = 20 nM). As in the case of its binding to Cdks, the indirubin derivative binds to GSK-3β's ATP-binding pocket. GSK-3β, along with Cdk5, is responsible for most of the abnormal hyperphosphorylation of the Tau observed in Alzheimer's disease (AD).
Indirubin-3′-monoxime-5-sulphonic acid	402088	1 mg	A potent inhibitor of Cdk1 (IC_{50} = 5 nM) and Cdk5 (IC_{50} = 7 nM). Also inhibits glycogen synthase kinase-3β (GSK-3β; IC_{50} = 80 nM). Similar to its binding to Cdks, the indirubin derivative binds to GSK-3β's ATP-binding pocket. GSK-3β, along with Cdk5, is responsible for most of the abnormal hyperphosphorylation of the microtubule-binding protein Tau observed in Alzheimer's disease (AD).
Tau, His•Tag®, Human, Recombinant, *E. coli*	580100	50 µg	Non-glycosylated and non-phosphorylated form of the 441-amino acid Tau splice variant. Tau promotes normal microtubule assembly and stabilizes microtubules *in vivo*. Hyperphosphorylated and glycated Tau tends to self-assemble and form paired helical filaments (PHFs), which are the basic structural components of neurofibrillary tangles (NFTs).

Note: For GSK-3 Inhibitors see pages 11-12

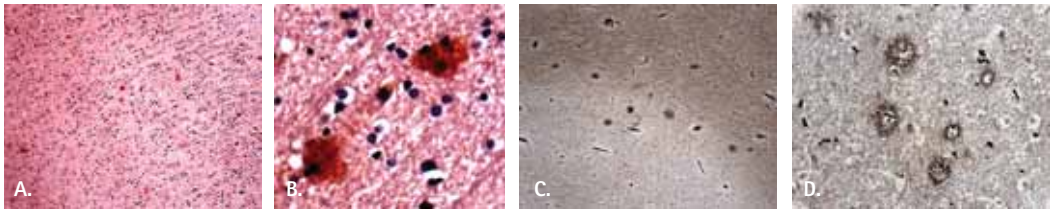
Alzheimer's Brain Tissue Sections

We are pleased to offer high-quality sections of human Alzheimer's brain tissues. These tissues have been fixed in freshly prepared paraformaldehyde, embedded in paraffin, sectioned, and mounted onto slides. Alzheimer's disease state has been validated by clinical symptoms, neuropathology reports, and staining for amyloid plaque accumulation and neurofibrillary tangles (see images below). Supporting data including patient history and neuropathology reports are available at www.novagen.com/alzheimers. Custom configuration is available upon request.

Product Name	Cat. No.	Size Price
Alzheimer's Brain Cerebral Cortex	71413	5 slides
Alzheimer's Brain Thalamus	71199	5 slides
Alzheimer's Brain Hippocampus	71200	5 slides

Product Name	Cat. No.	Size Price
Alzheimer's Brain Pons	71201	5 slides
Alzheimer's Brain Basal Ganglia	71202	5 slides
Alzheimer's Brain Cerebellum	71203	5 slides

Brain tissue sections on slides ready for in situ hybridization, immunohistochemistry, or laser capture microdissection.



Cerebral Cortex, Cat. No. 71413: Tissue stained for amyloid plaques (by Congo Red stain) showing amyloid deposition. **A.** 100X; **B.** 400X

Hippocampus, Cat. No. 71200: Tissue stained for neurofibrillary tangles (by Bielschowsky's method). **C.** 100X; **D.** 400X.

Spongiform Encephalopathies: The Deadly Folding of Prion Proteins

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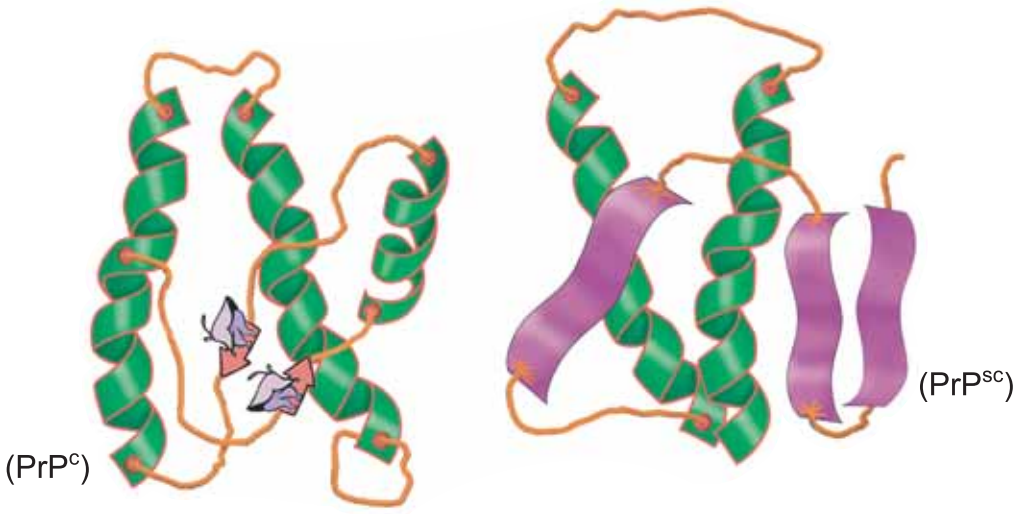

Prion diseases, collectively known as spongiform encephalopathies, are characterized by large vacuoles in the cortex and cerebellum. Typical symptoms of these diseases include loss of motor control, dementia, and paralysis. One form of this disease is known as Scrapie in sheep and bovine spongiform encephalopathy (BSE) in cows. In the early 1980's, Stanley Prusiner's group showed that Scrapie was caused by a protein; hence, the term "prion" was used to describe this atypical infectious agent. Evidence was presented showing that the prion is a small proteinaceous infectious particle that is resistant to the action of proteases. Kuru, a severe motor disease with symptoms of ataxia, was first described in an isolated tribe in New Guinea, where ingesting the brain tissue of dead relatives was established as the route of transmission. The pathological findings of Kuru were similar to encephalopathies found in the sheep brain. Creutzfeldt-Jakob disease (CJD), the human form of spongiform encephalopathies, is a rare but fatal neurodegenerative disorder also linked to abnormal prion proteins. CJD causes rapidly progressive dementia and neuromuscular disturbances. The duration of the disease, from the onset of symptoms to the inevitable death, is usually one year. The duration of the disease, the rarity of amyloid plaques, and the presentation of severe dementia can differentiate CJD from Kuru. Iatrogenic transmission of CJD has been reported in cases involving corneal transplants, implantation of electrodes in the brain, contaminated surgical instruments, and the injection of natural human growth hormone derived from cadaveric pituitaries.

Prion proteins have an apparent molecular weight of 33 - 35 kDa and exist in either PrP^{c} (c = cellular) or PrP^{sc} (sc = Scrapie) form. PrP^{c} is a glycoprotein normally found inserted in the plasma membrane by a glycosyl phosphatidylinositol (GPI) anchor and can be easily digested by proteases. It is composed mainly of α-helices. The PrP^{sc} has more of a β-sheet type structure and is highly resistant to the action of proteases. It accumulates intracellularly within cytoplasmic vesicles. The prion proteins possess two N-linked glycosylation sites and may contain over 50 different sugars. The large size of the N-linked sugars enables them to shield two orthogonal faces of the protein almost completely and protect large regions of the protein surface from proteases. The PrP^{sc} is shown to contain the same

set of glycans as PrP^c, but has a higher proportion of tri- and tetra-antennary sugars. It has been shown that, following proteinase K treatment, PrP^c is completely digested, whereas PrP^{sc} is only shortened to a 27 - 30 kDa fragment.

One of the most startling features of PrP^{sc} is its ability to multiply without nucleic acids. In prion diseases, the PrP^c form is post-translationally changed into the disease causing PrP^{sc} form. This involves a reduction of α-helix structures and an increase in β-sheet structures in the protein. When PrP^{sc} comes in contact with PrP^c, it converts more PrP^c into the PrP^{sc} form, forming large aggregates of "amyloid." Once PrP^c adopts the β-sheet structure of PrP^{sc}, it detaches from the cell membrane and is absorbed by vesicles within the cell. It begins to accumulate in lysosomes which swell up and rupture, releasing proteases and PrP^{sc} into the cell. As this process progresses simultaneously in multiple cells the entire brain gradually becomes riddled with dead and dying nerve cells, lending it the spongiform appearance that characterizes prion diseases. Morphologically, the amyloid of prion disease appears to be similar to that seen in Alzheimer's disease; however, the resulting brain pathologies are quite different. Prion-induced spongiform changes are not seen in brains of patients with Alzheimer's diseases.

Current research on prion proteins is focused on their ability to be transmitted from species to species. This is particularly alarming because of the recent outbreak of BSE in Great Britain. Although evolutionary proximity between cows and humans does not exist, prions from cows and humans do share some homology in the region that plays a role in the transmission of disease.

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Prion-Related Products

Product Name	Cat. No.	Size	Comments
	Applications	Price	
Ferric-Deuteroporphyrin IX, 2,4-Bis(ethylene glycol), Chloride	341390	10 mg	An iron-containing porphyrin that acts as an anti-TSE (anti-transmissible spongiform encephalopathy) agent. Inhibits the conversion of protease-sensitive to protease-resistant prion protein (IC_{50} ~1 µM) without any apparent cytotoxicity in scrapie-infected mouse neuroblastoma cells (ScNB) and in purified preparations. Suggested to bind to PrP with high affinity and cause conformational changes to the protein.
Ferric-Phthalocyanine Tetrasulfonate, Chloride	341395	25 mg	An iron-containing tetrapyrrole compound that is reported to inhibit the conversion of protease-sensitive prion protein (PrP-sen) to protease-resistant prion protein (PrP-res; IC_{50} ~ 0.9 µM) without any apparent cytotoxicity in scrapie-infected mouse neuroblastoma cells (ScNB) and in purified protein preparations. Suggested to bind to PrP with high affinity and cause conformational changes to the protein.
Phthalocyanine Tetrasulfonate	526150	25 mg	A tetrapyrrole compound that acts as an anti-TSE (anti-transmissible spongiform encephalopathy) agent. Blocks the conversion of protease-sensitive prion protein (PrP-sen) to protease-resistant prion protein (PrP-res; IC_{50} < 1 µM) without any apparent cytotoxicity in scrapie-infected mouse neuroblastoma cells (ScNB). Also blocks the conversion of PrP-sen to PrP-res in purified protein preparations. Suggested to bind to PrP with high affinity and cause conformational changes.
Prion Peptide (106-126), Human	529602	500 µg	A 21-residue peptide containing the "toxic" core of Scrapie prion protein (PrP^{sc}) that mimics the physio-chemical properties of PrP^{sc}. Adopts a prevalent β-sheet structure that is partially resistant to proteolysis. Induces the synthesis of transmembrane prion protein. Displays neurotoxicity and induces microglial activation. Also reported to induce apoptosis via mitochondrial disruption.

Prion-Related Products, cont.

Product Name	Cat. No. Applications	Size Price	Comments
Prion Protein, His•Tag®, Bovine, Recombinant, *E. coli*	**530005** —	100 µg	A full-length mature part of bovine PrP (25-244) expressed in *E. coli*. The cellular isoform of the prion protein (PrP^{C}) is a glycosylphosphatidylinositol-anchored glycoprotein, normally expressed in neural and non-neural tissues, including skeletal muscle. In transmissible spongiform encephalopathies (TSE) or prion diseases, PrP^{C}, which is soluble in non-denaturing detergent and sensitive to proteinase K (PK) treatment, represents the molecular substrate for the production of a detergent-insoluble and PK-resistant isoform, called PrP^{Sc} (prion scrapie). The interaction of PrP^{Sc} with PrP^{C} causes the abnormal structural conversion of the latter resulting in TSE.
Prion Protein, His•Tag®, Human, Recombinant, *E. coli*	**530006** —	100 µg	A full-length mature part of human prion protein (23-231) expressed in *E. coli*. Useful as an antigen standard in immunochemical detection of Creutzfeldt-Jakob Disease (CJD).
Anti-Prion Protein (PrP) (Ab-1), Human (Rabbit)	**PC360** FS, IB	100 µl	Detects PrP^{c} 33 - 35 and PrP^{sc} 27 - 30 in normal and infected sheep brain.
Anti-Prion Protein (PrP) (Ab-2), Hamster (Rabbit)	**PC404** IB, IH **PC404T** IB, IH	100 µl 10 µl	Detects PrP^{c} 33 - 35 and PrP^{sc} 27 - 30 in normal and infected sheep brain.
Anti-Prion Protein (PrP) (Ab-3), Bovine (Mouse)	**NB36** ELISA, FS, IB, IH **NB36T** ELISA, FS, IB, IH	200 µg 10 µg	Recognizes both PrP^{c} and PrP^{sc} by ELISA. In Western blots, recognizes a 33 - 35 kDa protein in normal animals and a 27 - 30 kDa protein which represents PrP in protease-treated brain extracts from infected animals.
Anti-Prion PrP27-30, N-Terminal (79-97), Human (Goat)	**530000** ELISA, IH	100 µl	Recognizes amyloid plaques in paraffin sections from CJD brains. The prion protein is conserved in many species.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FS:** Frozen Sections; **IB:** Immunoblotting; **IH:** Immunohistochemistry; **IP:** Immunoprecipitation; **PS:** Paraffin Sections

Parkinson's Disease: α-Synuclein, A Perpetrator of Disease

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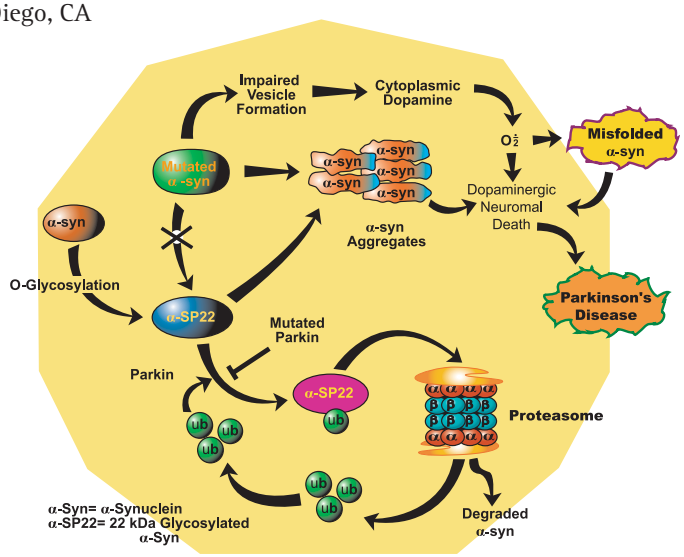

Parkinson's disease (PD), the second most common neurodegenerative disease, is a progressive neurological condition caused by the degeneration of dopaminergic neurons in the substantia nigra (SN) in the midbrain region. The etiology of the disease is not completely understood, however, inherited risk factors and environmental toxins are considered likely causes. A search for genetic causes of PD have shown four independent gene loci in various forms of familial PD. The physical symptoms of PD include tremors, rigidity, and bradykinesia.

PD and certain other dementias are associated with brain lesions known as Lewy bodies, which contain α-synuclein (α-Syn) as the major component. Mutations in the α-Syn gene have been linked with certain familial forms of PD. It has been suggested that mutations in α-Syn (Ala^{53} to Thr^{53} and Ala^{30} to Pro^{30}) may cause a conformational change that renders α-Syn more prone to self-aggregation and deposition in Lewy bodies. Expression of mutant α-Syn in dopaminergic neurons impairs synaptic vesicle formation, increases cytoplasmic levels of dopamine, and increases superoxide radicals in the cytoplasm, which lead to oxidative stress and misfolding of α-Syn. Although much is known about the aggregation α-Syn, little information is available on its degradation. Neurosin (kallikrein-6), a serine protease, which degrades α-Syn, is shown to co-localize with Lewy bodies. *In vitro* studies have shown that neurosin prevents α-Syn polymerization by reducing the amount of monomer and also by generating fragmented α-Syn. Under cellular stress, neurosin is released from mitochondria to the cytosol, which results in the increase of degraded α-Syn species.

Various mutations in yet another gene, the Parkin gene, are reported in early autosomal-recessive form of PD, however, these mutations do not generate Lewy bodies. The Parkin gene's product is an E3 ubiquitin ligase. Known substrates of Parkin include Pael-R (Parkin-associated endothelin-receptor-like receptor), CDCrel-1 (cell-division-control-related protein 1), Synphilin-1, and a glycosylated form of α-Syn (α-SP22). Only the glycosylated form, α-SP22, is ubiquitinated by Parkin. Unfolding of Pael-R makes it insoluble and allows it to accumulate in the endoplasmic reticulum. Ubiquitination of Pael-R by Parkin leads to its degradation in the proteasome, however, failure to ubiquitinate it leads to the death of the neuron.

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Parkin Cleavage Inhibitor

(Ac-Leu-His-Thr-Asp-CHO)

Cat. No. 512660 1 mg
5 mg

A tetrapeptide aldehyde corresponding to the Parkin amino acid sequence 123 - 126 (putative scissile peptide bond Asp^{126} - Ser^{127}) that acts as an efficient inhibitor of apoptosis-associated parkin cleavage in SH-SY5Y and CHO cells. *Purity: ≥95% by HPLC.*

Ref.: Kahns, S., et al. 2002. *J. Biol. Chem.* **277,** 15303.

Synucleins and Related Products

Product Name	Cat. No.	Size Price	Comments
R-(—)-Deprenyl, Hydrochloride	262000	100 mg	An irreversible, selective monoamine oxidase-B (MAO-B) inhibitor (IC_{50} = 17 nM) with neuroprotective and vasodilatory properties. Has only a trivial effect on MAO-A enzyme (IC_{50} > 100 µM). Useful for the treatment of Parkinson's disease and to delay the progression of Alzheimer's disease. Protects cells from apoptosis induced by reactive oxygen species and peroxynitrite. Induces rapid increase in nitric oxide production in brain tissue. *In vivo*, it rescues nigral dopaminergic neurons after systemic MPTP treatment. Induces a transient inflow of Ca^{2+} through the voltage-dependent N-type Ca^{2+} channel. Also reported to inhibit the activity of calmodulin-dependent phosphodiesterase in bovine brain.
α-Synuclein, Human, Recomb., *E. coli*	575001	200 µg	α-Synuclein is an acidic neuronal protein of 140 amino acids (amino acid residues 1-140). This enzyme has been implicated in the pathogenesis of Parkinson's Disease and related neurodegenerative disorders, and more recently, to be an important regulatory component of vesicular transport in neuronal cells.
α-Synuclein A30P, Human, Recomb., *E. coli*	575002	200 µg	A point mutant (A30P) of the α-Synuclein gene that has been linked to autosomal dominant early onset Parkinson's Disease (PD).
α-Synuclein A53T, Human, Recomb., *E. coli*	575003	200 µg	A point mutant (A53T) of the α-Synuclein gene that has been linked to autosomal dominant early onset Parkinson's Disease (PD).
α-Synuclein (ΔNAC), Human, Recomb., *E. coli*	575004	100 µg	A deletion mutant of α-Synuclein that lacks the non-αβ component (NAC; amino acid residues 61-95). Does not bind to αβ1-38 whereas the precursor of the non-αβ component of Alzheimer's disease amyloid (NACP) does bind to αβ1-38.
α-Synuclein A30P/A53T, Human, Recomb., *E. coli*	575005	200 µg	A Parkinson's disease-related double mutant (A30P/A53T) of α-Synuclein.
α-Synuclein (1-60), Human, Recomb., *E. coli*	575009	100 µg	A deletion mutant of α-Synuclein that contains the N-terminal amphipathic domain (amino acid residues 1-60).
α-Synuclein (61-140), Human, Recomb., *E. coli*	575006	100 µg	A deletion mutant of α-Synuclein (amino acid residues 61-140).
α-Synuclein (96-140), Human, Recomb., *E. coli*	575007	100 µg	A deletion mutant of α-Synuclein (amino acid residues 96-140).
γ-Synuclein, Human, Recomb., *E. coli*	575008	200 µg	γ-Synuclein is an acidic neuronal protein of 127 amino acids. It is up-regulated in the majority of late-stage breast and ovarian cancers. It has also been shown that there is a correlation between γ-Synuclein expression in breast ductal carcinomas and the staging of the cancer, suggesting that γ-Synuclein may be a potential marker for both late stage breast and ovarian cancers. More recently, it has been shown that γ-Synuclein promotes cancer cell survival and inhibits stress and chemotherapy drug-induced apoptosis by modulating MAP kinase pathways.

Dopamine Receptor Antagonists and Agonists

Product Name	Cat. No.	Size Price	Comments
Bromocriptine Mesylate	203850	25 mg	Dopamine D_2 receptor agonist that potently and selectively inhibits neuronal NOS (nNOS; IC_{50} = 10 µM) by affecting the activation of NOS by calmodulin. Inhibits Mg^{2+} ATPase activity (IC_{50} = 300 nM) and verapamil-induced P-glycoprotein ATPase activity (K_i = 200 nM).
Carmoxirole	217510	5 mg	A 5-carboxyindole-3-butylamine analog that acts as a selective, presynaptic dopamine D_2-receptor agonist.
EMD 23448	324651	5 mg	An indolyl-3-butylamine analog that acts as a potent dopamine autoreceptor agonist *in vitro* and *in vivo*. Active at supersensitive postsynaptic D_2-receptors with virtually no activity at the normo-sensitive postsynaptic D_2-receptors.
Haloperidol	371980	100 mg	Dopaminergic receptor antagonist that increases dopamine release by blocking presynaptic D_2 receptors. Has only a weak postsynaptic effect.
3-Hydroxytyramine, Hydrochloride	4000	25 g	Dopaminergic neurotransmitter. Precursor of adrenaline and noradrenaline. Dopamine activity is mediated via its action on D_1 and D_2 receptors. Stimulation of D_1 receptors activates adenylate cyclase, while D_2 receptor activation inhibits adenylate cyclase.
3-Methoxytyramine, Hydrochloride	45426	100 mg	A metabolite of dopamine. Useful as a marker of dopamine release.
Piribedil, Maleate	527900	10 mg	An anti-parkinsonian agent. A direct dopamine receptor agonist that displays 20-fold higher affinity for dopamine D_3 than for dopamine D_2-like receptors, and very low affinity for the dopamine D_1 receptor subtype.
Roxindole Mesylate	557375	5 mg	A selective dopamine (DA) D_2-receptor agonist. Possesses anti-depressant and anti-parkinsonian activity.
L-Stepholidine, *Stephania intermedica*	569403	20 mg	Extremely potent dopamine receptor antagonist with higher affinity for D_1 receptors over D_2 receptors. Has 18-fold greater potency than haloperidol for D_1 receptor binding and 14-fold weaker potency for D_2 receptor binding. Also acts as a Ca^{2+} channel blocking agent (IC_{50} = 18.1 µM).
Tetrahydropalmatine, Hydrochloride	584218	5 mg	A D_1/D_2 dopaminergic receptor antagonist with anti-arrhythmic and hypotensive properties. Has analgesic and sedative/hypnotic effects.
U-99194A	662059	25 mg	A D_3 dopamine receptor antagonist with a 30-fold preference for the D_3 vs. D_2 dopamine receptor.

Antibodies for Parkinson's Disease Research

Product Name	Cat. No. Applications	Size Price	Comments
Anti-DOPA Decarboxylase, Bovine (Rabbit)	324382 IB, IH	30 µg	Reacts with bovine and rat. Shows weak reactivity with human and monkey.
Anti-DOPA Decarboxylase, Human (Rabbit)	324381 IB, IH	30 µg	Reacts with bovine, canine, human, rabbit, and sheep. Does not react with rat.
Anti-Dopamine β-Hydroxylase, C-Terminal, Human (Sheep)	324383 IB,IH	30 µg	Reacts with bovine, human, and monkey. Does not react with canine, rat, and sheep.
Anti-Dopamine β-Hydroxylase, N-Terminal, Human (Sheep)	324384 IB	40 µg	Reacts with bovine, canine, human, rabbit, rat, and sheep. Does not react with monkey.
Anti-Dopamine (Rabbit)	324379 ELISA, IH	50 µl	Reacts with a wide range of species.
Anti-Dopamine D_1 Receptor, Human (Rabbit)	324390 ELISA, IB, IC	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors.
Anti-Dopamine D_2 Receptor, Human (Rabbit)	324393 ELISA, IB, IC	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors and minimal cross-reactivity with the D2S short receptor.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IH:** Immunohistochemistry

Antibodies for Parkinson's Disease Research, cont.

Product Name	Cat. No. Applications	Size Price	Comments
Anti-Dopamine D_{2S} Receptor, Human (Rabbit)	324396 ELISA, IB, IH	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors.
Anti-Dopamine D_3 Receptor, Human (Rabbit)	324402 ELISA, FC, IB, IH	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors.
Anti-Dopamine D_4 Receptor, Human (Rabbit)	324405 ELISA, IB, IC	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors.
Anti-Dopamine D_5 Receptor, Human (Rabbit)	324408 ELISA, IB, IC	100 µl	Reacts with human and rat. Does not cross-react with other dopamine receptors.
Anti-Dopamine β-Hydroxylase, C-Terminal, Human (Sheep)	324383 IB, IH	30 µg	Reacts with bovine, human, and monkey. Does not cross-react with canine, rat, and sheep dopamine β-hydroxylase by immunoblotting.
Anti-Parkin, Human (Rabbit)	PC372 FS, PS PC372T	25 µg 5 µg	Reacts with human, mouse, and rat.
Anti-Parkin, N-Terminal (83-97), Human (Goat)	512650 ELISA, IB, PS	100 µl	Reacts with 65 kDa human Parkin.
Anti-α-Synuclein, C-Terminal (116-131), Human (Goat)	575000 ELISA, PS	100 µl	Recognizes α-synuclein and labels Lewy bodies in Parkinson's brain tissue paraffin sections.

Key: ELISA: Enzyme-Linked Immunosorbent Assay; **FC:** Flow Cytometry; **FS:** Frozen Sections; **IB:** Immunoblotting; **IC:** Immunocytochemistry; **IH:** Immunohistochemistry; **PS:** Paraffin Sections

Miscellaneous

Product Name	Cat. No.	Size Price	Comments
Clioquinol	233165	1 g	A neurotoxic antibiotic that is reported to dissolve senile plaques and reduce amyloid's ability to clump together.
Human Alzheimer's Brain cDNA Library	70655	100 µl	Bacteriophage λ library constructed from human Alzheimer's brain cDNA. cDNAs were cloned using a proprietary directional random priming method and supplied as a 100 µl aliquot of phage containing > 5 x 10^6 primary clones (enough for 50-500 screenings). Information regarding appropriate host strains, protocols for titering, screening, and auto-subcloning is provided.
Proteasome Substrate IV, Fluorogenic (Z-VKM-AMC)	539143	5 mg	Fluorogenic substrate for Alzheimer's disease amyloid A4-splitting enzymes and for the proteasome. Ex. max.: ~ 380 nm; Em. max.: ~ 460 nm.
TPEN	616394	100 mg	Lipid-soluble heavy metal ion chelator useful in extracting Aβ amyloid deposits from Alzheimer's disease brain tissue. Has high affinity for heavy metals but low affinity for Ca^{2+} and and Mg^{2+}.
Riluzole	557324	50 mg	A cell-permeable inhibitor of glutamate release. A polyglutamine aggregation inhibitor that stimulates the synthesis of NGF, BDNF, and GDNF in mouse astrocytes. Offers neuroprotection and slows the progression of amyotrophic lateral sclerosis and prolongs the lifespan of animal models of Huntington's disease.

Prices and availability are subject to change without notification.

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