Antibody Sampler Kits For Neuroscience Research



Toll-Free Tel: (US & Canada): 1.877.BIOLEGEND (246.5343) Tel: 858.768.5800 biolegend.com

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BioLegend®

Antibody Sampler Kits for Neuroscience Research

BioLegend seeks to advance research discoveries in Neuroscience. We now offer a variety of antibody sampler kits that provide flexibility for sampling and detection of key cellular and synaptic markers, targets in neurodegeneration, and protein degradation machinery. These sampler kits contain 25 µg sizes of antibodies that are highly suitable for western blotting (WB), immunohistochemistry (IHC), and immunocytochemistry (ICC) as indicated on their data sheets.

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ΑΡΡ/Αβ

Amyloid precursor protein (APP) is a transmembrane protein that is highly expressed in neuronal tissues and enriched at synapses. APP undergoes proteolytic processing through two distinct pathways: 1) the amyloidogenic pathway that generates amyloid beta (A β) peptides through sequential processing by β - and γ -secretases, and 2) the non-amyloidogenic pathway, where processing by α -secretase precludes the generation of A β . Aberrant processing and accumulation of A β peptides leads to the formation of oligomeric and fibrillar aggregates, which interfere with cellular processes and lead to neuronal cell death. The A β aggregates are a hallmark of Alzheimer's disease (AD) and other A β -related disorders, and can be used as a tool for pathology detection.

APP/Aβ Antibody Sampler Kit

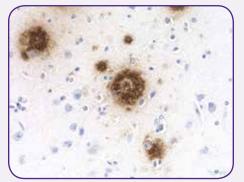
BioLegend's APP/A β Antibody Sampler Kit offers an easy solution for detection of full-length APP and various APP cleavage fragments including A β 1-40 and A β 1-42 peptides. This kit includes clones with unique advantages. In contrast to other gold standard antibodies for A β detection, such as clones 6E10 and 4G8, clone 3A1 demonstrates preference for aggregated forms of A β and shows no cross-reactivity with APP. Clone M3.2 is rodentspecific and recognizes A β , β -CTF, sAPP- α , and full-length APP. Clone M3.2 has negligible cross-reactivity with human isoforms of APP and A β . Clone 12F4 reacts only with A β 1-42 peptide while clone 11A50-B10 shows reactivity towards A β 1-40.

APP/Aβ Antibody Sampler Kit Contents:

Clone	Format	Reactivity	Application
6E10	Purified	Hu	WB, IHC-P, Direct ELISA
M3.2	Purified	Ms, Rat	WB
11A50-B10	Purified	Hu	IHC-P
12F4	Purified	Hu, Ms, Rat	WB, IHC-P, Direct ELISA
3A1	Purified	Hu	IHC-F, Direct ELISA
	6E10 M3.2 11A50-B10 12F4	6E10PurifiedM3.2Purified11A50-B10Purified12F4Purified	6E10PurifiedHuM3.2PurifiedMs, Rat11A50-B10PurifiedHu12F4PurifiedHu, Ms, Rat

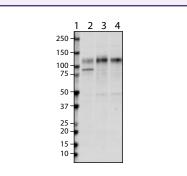
Learn more about APP and Aβ detecting clones at: biolegend.com/amyloid precursor protein

Amyloid β-1-40



IHC staining of purified anti- β -Amyloid, 1-40 antibody (clone 11A50-B10) on FFPE Alzheimer's disease brain tissue. The section was counterstained with hematoxylin.

Nicastrin



Western blot of purified anti-Nicastrin antibody (clone 9C3). Lane 1: Molecular weight marker; Lane 2: 20 µg of human brain lysate; Lane 3: 20 µg of mouse brain lysate; Lane 4: 20 µg of rat brain lysate.

β/γ Secretase

Secretases are proteolytic enzymes involved in the processing of A β . Beta-secretase, also known as beta-site APP cleavage enzyme 1 (BACE1), is a protease expressed in neurons. The cleavage of APP at the N-terminal of the A β domain by BACE1 releases a soluble extracellular fragment called sAPP β . The remaining portion of APP, which is membrane-bound, is termed C-terminal fragment (C99).

Gamma-secretase is a multi-subunit protease complex consisting of nicastrin, presenilin-1/2 (PSEN1/2), presinilin enhancer 2 (PEN-2), and anterior pharynx-defective 1 (APH-1). The components of this complex are widely expressed in tissues including the brain. This complex is responsible for the processing of the C99 fragment to release A β isoforms, including A β 1-40 and A β 1-42, and a fragment known as the APP intracellular domain (AICD). Increased A β production has been partly attributed to mutations in the APP or PSEN genes, which occur in familial forms of AD. These mutations favor the processing of APP by β - and γ -secretases leading to elevated levels of A β .

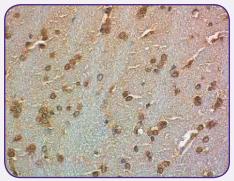
β/γ Secretase Antibody Sampler Kit

The β/γ Secretase Antibody Sampler Kit includes essential components of the secretase proteolytic machinery including BACE1, nicastrin, PSEN1, and PSEN2. The β/γ secretase proteins are often found associated with cellular membranes such as the endosome and the plasma membrane. These antibodies are therefore ideal for detection of protein expression levels and localization within cellular compartments. The tubulin β 3 antibody was included as a loading control for WB or visualization of neurons by immunostaining.

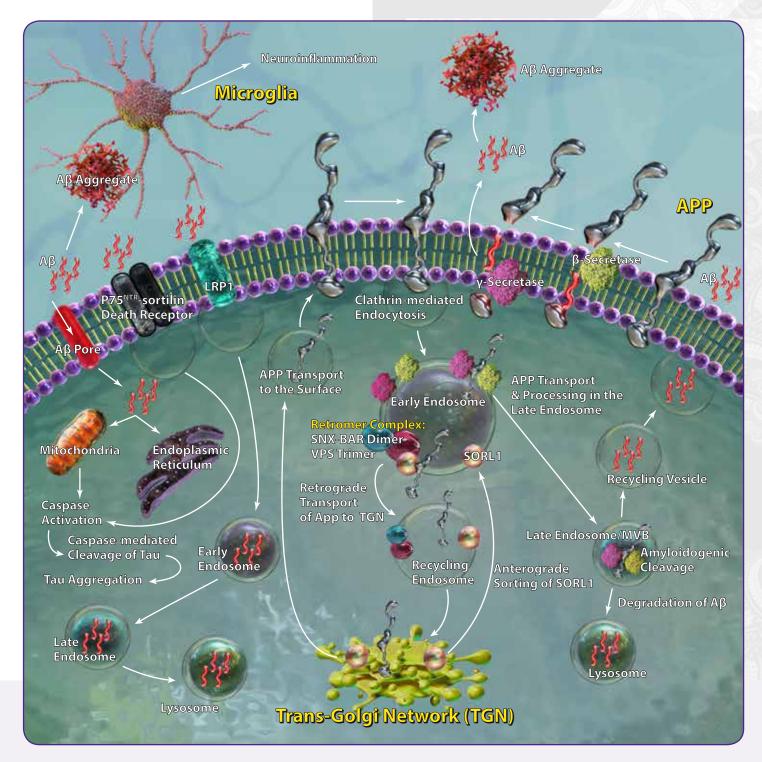
β/γ Secretase Antibody Sampler Kit Contents:

		•		
Specificity	Clone	Format	Reactivity	Application
BACE1	A17035K	Purified	Hu	WB, Direct ELISA
Nicastrin	9C3	Purified	Hu, Ms, Rat	IHC-P, WB
Presenilin 1 (N terminus)	NT1	Purified	Hu	WB
Presenilin 2	PS2	Purified	Hu	WB
Tubulin β 3 (TUBB3)	TUJ1	Purified	Hu, Ms, Rat	IHC-P, ICC, WB

Nicastrin



IHC staining of purified anti-Nicastrin antibody (clone 9C3) on FFPE rat brain tissue. The section was counterstained with hematoxylin.



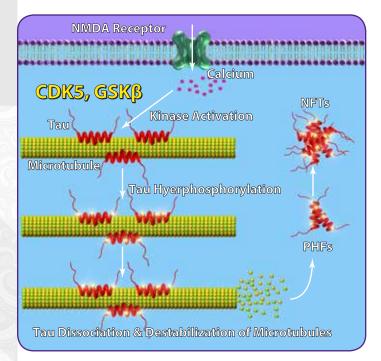
APP processing and trafficking in a cell. The newly synthesized APP protein is transported from the trans-golgi network (TGN) to the plasma membrane (PM) via the endocytic pathway. Under disease conditions, APP is sequentially processed by β - and γ -secretases at the PM to generate A β fragments. APP can undergo clathrin-mediated endocytosis and is recycled back to the TGN. Sortilin-related receptor L (DLR class) A, also known as SORL1, acts as sorting receptor for APP by shuttling between the TGN and early endosomes, and interacting with the retromer complex for retrograde sorting of APP into the recycling endosomes. Amyloidogenic processing of APP can also occur in endosomes, where a subset of A β peptides are transported to the lysosomes for degradation or recycled back to the surface. Extracellular A β oligomers mediate the formation of p75NTR-sortilin death receptor, as well as creating pores in the PM, resulting in caspase activation and caspase-mediated cleavage and aggregation of Tau. A β can also bind to low density lipoprotein receptor-related protein 1 (LRP1), endocytosed through the clathrin-dependent pathway, and trafficked to, and degraded in the lysosomes. Recognition of A β oligomers and amyloid plaques by microglia can trigger persistent neuroinflammation, activating signaling pathways that lead to cell death.

Tau

Tau protein is expressed abundantly in neurons and has a number of functions in the central nervous system. Tau binds to microtubules and leads to their assembly, stabilization, and maintenance. Tau also regulates motor-driven axonal transport. Phosphorylation of Tau is a common post-translational modification that plays an important role in the solubility, localization, and function of Tau. Conformational changes in Tau have been linked to excessive phosphorylation of this protein, and decreased microtubule binding and stability. Hyperphosphorylated Tau has a propensity to accumulate and form protein aggregates in neurons. These aggregates eventually form intracellular filamentous inclusions, known as neurofibrillary tangles (NFTs), that are detected biochemically and immunohistologically in neurodegenerative disorders termed Tauopathies. Hyperphosphorylated and aggregated Tau interferes with normal neuronal function, such as microtubule dynamics, and ultimately leads to neurodegeneration.

Tau Antibody Sampler Kit

The Tau Antibody Sampler Kit offers several advantages for those interested in studying the phosphorylation status and/ or expression levels of different Tau isoforms. Tau exists in six isoforms that are differentially expressed during development. These isoforms are distinguished by the number of tubulin binding domains, 3 (3R) or 4 (4R), in the C-terminal of the protein and by one (1N), two (2N), or no (0N) inserts in the N-terminal domain. Clones A16103A and A16097F cross-react with human 3R and 4R isoforms and show minimal to no reactivity with murine Tau protein. Clones A15091A and M7004D06 recognize phosphorylated Tau at residues serine 262 and threonine 181, respectively. These phosphorylated forms are often detected in Tau lesions. Measurement of cerebrospinal fluid phospho-Tau 181 may also have diagnostic utility for several neurological disorders including AD. Antibodies against these sites can be used to visualize classical tau pathologies such as NFTs, neuropil threads, and neuritic plaques, by immunohistochemistry.



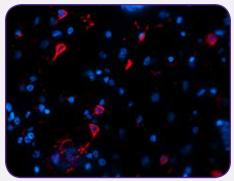
Calcium-mediated phosphorylation of Tau in AD. Prolonged NMDA-dependent calcium influx in AD leads to overactivation of kinases such as CDK5 and GSKβ, which in return hyperphosphorylate Tau resulting in Tau dissociation and destabilization of microtubules. Hyperphosphorylated Tau has a propensity for aggregation, forming paired helical filaments (PHFs) and neurofibrillary tangles that disrupt intracellular transport and ultimately cause neurodegeneration.

Tau Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
Tau Phospho (Ser262)	A15091A	Purified	Hu	IHC-P, Direct ELISA
Tau Phospho (Thr181)	M7004D06	Purified	Hu	IHC-P, WB, Direct ELISA
Tau, 1-223	A16103A	Purified	Hu	IHC-P, WB, Direct ELISA
Tau, 368-441	A16097F	Purified	Hu	IHC-P, WB, Direct ELISA

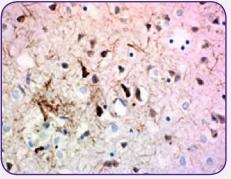
Learn more about pan- and isoform-specific Tau antibodies and recombinant proteins at: <u>biolegend.com/tau</u>

Tau Phospho (Thr181)



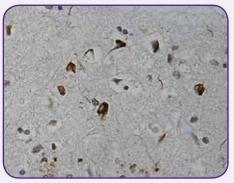
IHC staining of purified anti-Tau phospho (Thr181) antibody (clone M7004D06, red) on FFPE Alzheimer's disease brain tissue. Nuclei were counterstained with Hoechst 33342 (blue).

Tau, 368-441



IHC staining of anti-Tau, 368-441 antibody (clone A161097F) on formalin-fixed FFPE Alzheimer's disease brain tissue. The section was counterstained with hematoxylin.

Tau Phospho (Ser262)



IHC staining of purified anti-Tau Phospho (Ser262) antibody (clone A15091A) on FFPE human Alzheimer's disease brain tissue. The section was counterstained with hematoxylin.

a-Synuclein

α-Synuclein has emerged as a biomarker for Parkinson's disease (PD) and a number of other α-Synuclein related disorders collectively termed as Synucleinopathies. α-Synuclein serves as a common link between inherited and sporadic forms of PD, as this protein and its post-translationally modified (PTM) forms are found in large and insoluble proteinaceous deposits known as Lewy bodies (LBs) and Lewy neurites. These PTMs, including phosphorylation and truncation, have been shown to promote aggregation and oligomerization of α-Synuclein, and play an important role in the pathogenesis of PD.

Using immunohistochemical and biochemical approaches in combination with phospho-specific antibodies, phosphorylated α -Synuclein at serine residue 129 (pS129) was shown to make up the majority of this protein in inclusions isolated from the postmortem tissues derived from patients diagnosed with PD and other Synucleinopathies. Therefore, pS129 has emerged as a hallmark of pathology in these disorders. Studies from PD-like animal and cell culture models, as well as further investigations into the constituents within LBs, have shown additional α -Synuclein phospho-species such as phosphorylated residues tyrosine 39 and serine 87. Biochemical and mass spectrometry analyses of α -Synuclein derived from LBs have also detected C-terminal truncated forms of this protein including a species that terminates at asparagine 122¹.

a-Synuclein Antibody Sampler Kit

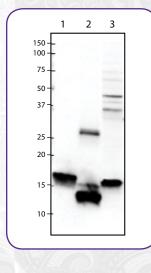
In line with these findings, BioLegend offers a number of antibodies that recognize native and modified forms of α-Synuclein. Clones P-syn/81A and A15119B are specific for and only recognize pS129 and pY39 species of α-Synuclein. Clone A15115A cross-reacts with the native and pS87, while clones A15127A and A15126D react with truncated forms of this protein. Refer to the TDS for α-Synuclein Antibody Sampler Kit to learn more about antibody specificities and applications in which these antibodies can be utilized.

a-Synuclein Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
α-Synuclein, 80-96	A15115A	Purified	Hu	IHC-P, WB, Direct ELISA
α-Synuclein, 117-122	A15126D	Purified	Hu	IHC-P, WB, Direct ELISA
α-Synuclein (C-Terminal Truncated x-122)	A15127A	Purified	Hu	IHC-P, Direct ELISA
α-Synuclein Phospho (Tyr39)	A15119B	Purified	Hu	IHC-P, Direct ELISA
α-Synuclein Phospho (Ser129)	P-syn/81A	Purified	Hu	IHC-P

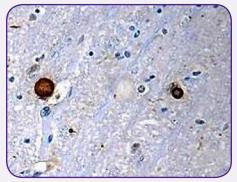
Learn more about α-Synuclein and PD at: biolegend.com/parkinsons_disease

a-Synuclein, 117-122



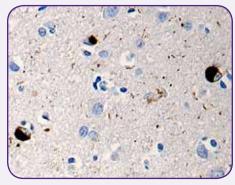
Western blot of anti-α-Synuclein antibody (clone A15126D). Lane 1: 50 ng of recombinant human α-Synuclein; Lane 2: 50 ng of recombinant C-terminally truncated human α-Synuclein (1-122); Lane 3: 20 µg of normal human brain lysate.

α-Synuclein (C-Terminal Truncated x-122)



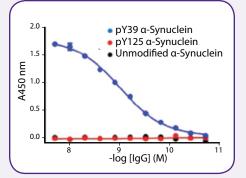
IHC staining of purified anti- α -Synuclein, C-Terminal Truncated antibody (clone A15127A) on FFPE Parkinson's disease brain tissue. The section was counterstained with hematoxylin.

a-Synuclein Phospho (Ser129)



IHC staining of purified anti-α-Synuclein Phospho (Ser129) antibody (clone P-syn/81A) on FFPE human Parkinson's disease brain tissue. The section was counterstained with hematoxylin.

a-Synuclein Phospho (Tyr39)



Direct ELISA of purified anti- α -Synuclein Phospho (Tyr39) antibody (clone A15119B) binding to plate-immobilized recombinant human unmodified, pY39, and pY125 α -Synuclein proteins.

Neuron Markers

Neurons are highly specialized cells with unique compartments that are distinguishable using specific markers. These compartments can be generally classified into soma (cell body), axon, dendrite, and synapse. Certain markers allow differentiation of neurons from other cell types in the nervous system, namely microglia, astrocytes, and oligodendrocytes (ODs). The use of antibodies for these markers in conjunction with microscopy serves as a powerful method for collecting data relevant for, but not limited to: 1) cell type identification, 2) cellular co-localization, 3) phenotypic and morphological analysis, and 4) protein expression levels. To this end, BioLegend offers a number of antibodies against cell type specific and structural markers that have been validated for use in IHC and/or ICC.

Neuron Marker Antibody Sampler Kit

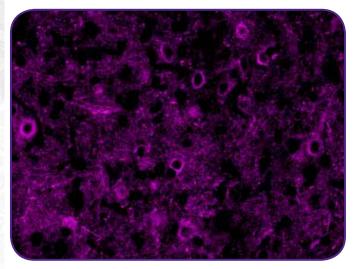
Our Neuron Marker Antibody Sampler Kit includes antibodies for markers expressed in different compartments of a neuron. Clone NSE-P1 detects Enolase 2, also known as NSE, which is a soluble protein used for identification of neurons and cells of neuronal origin. This antibody can be used to visualize the soma and neuronal processes. Clone 1B7 was raised against human Neuronal Nuclei (NeuN) protein, also known as Fox3. This antibody reveals strong nuclear staining of a wide range of neuronal cell types. There are some neuronal cells that are not detected by NeuN, such as Purkinje neurons, Golgi cells, and retinal photoreceptor cells. Clone SMI 52 reacts with the structural protein microtubule-associated protein 2 (MAP2), and recognizes neuronal cell bodies and dendrites in tissue sections and cell cultures. Clone TUJ1 is highly reactive to class III β-tubulin, another cytoskeletal protein expressed in neurons. Clone TUJ1 does not react with β -tubulin found in glial cells. Immunostaining with TUJ1 allows visualization of cell bodies, dendrites, and axons.

Neuron Marker Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
Neurofilament H (NF-H), Nonphosphorylated	SMI 32	Alexa Fluor® 594	Hu, Ms, Rat	IHC-P, ICC, IHC-F
MAP2	SMI 52	Alexa Fluor® 488	Ms, Rat	IHC-P
NSE	NSE-P1	Alexa Fluor® 647	Hu, Ms, Rat	IHC-P, WB
FOX3 (NeuN)	1B7	Purified	Hu, Ms, Rat	IHC-P, WB
Tubulin β 3 (TUBB3)	TUJ1	Alexa Fluor [®] 594	Hu, Ms, Rat	IHC-P, ICC, WB

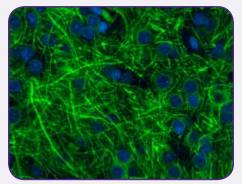
Download our Neuronal Cell Markers poster to learn more about our selection of antibodies for neuronal compartments at: <u>biolegend.com/literature</u>

NSE



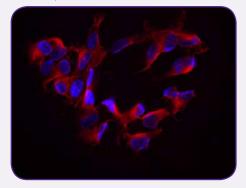
IHC staining of Alexa Fluor* 647 anti-NSE antibody (clone NSE-P1) on FFPE mouse brain tissue.

MAP2



IHC staining of Alexa Fluor® 488 anti-MAP2 antibody (clone SMI 52, green) on FFPE rat brain tissue. Nuclei were counterstained with DAPI (blue).

Tubulin β3



ICC staining of Alexa Fluor[®] 594 anti-Tubulin β 3 (TUBB3) antibody (clone TUJ1, red) on SH-SY5Y neuroblastoma cells. Nuclei were counterstained with Hoechst 33342 (blue).

NeuN



IHC staining of purified anti-FOX3 (NeuN) antibody (clone 187) on FFPE mouse brain tissue. The section was counterstained with hematoxylin.

Neurofilament L/M/H

Neurofilaments (NFs) belong to the intermediate filament family of proteins, and are primarily composed of three subunits; neurofilament light (NF-L), medium (NF-M), and heavy (NF-H). NFs are essential for providing structural support and maintenance of axon caliber. Filament assembly, function, and molecular interactions of neurofilaments can be regulated by phosphorylation. In AD patients, aberrant phosphorylation of NF-M and NF-H leads to their accumulation in neuronal cell bodies, and disruption of NF transport to the axons. This has been attributed to decreased phosphatase and elevated NF kinase activity in the diseased brains. NFs can also be released from damaged or diseased neurons into blood or CSF. Therefore, elevated levels of NFs in the serum or CSF can serve as a biomarker for neuronal injury or degeneration. Indeed, elevated CSF levels of NF-L and NF-H have been reported in AD or other dementias²

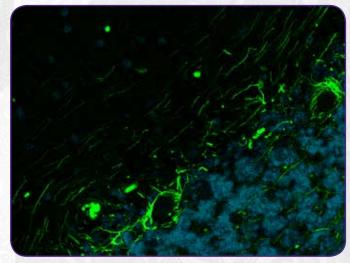
Neurofilament L/M/H Antibody Sampler Kit

BioLegend's Neurofilament L/M/H Antibody Sampler Kit offers a great selection of highly validated antibodies for detection of neurofilament subunits. These antibodies are offered in Alexa Fluor[®] conjugated formats to allow multiplex immunofluorescence (IF) staining in tissue or cell culture. The kit components allow visualization of neuronal axons, dendrites, and cell bodies depending on the clone utilized. Clone SMI 31 detects a phosphorylated epitope in extensively phosphorylated NF-H and primarily reacts with axons. Clone SMI 32 detects a non-phosphorylated epitope in NF-H and visualizes neuronal cell bodies, axons, and dendrites. Clones SMI 35 and SMI 310 generally react with axons. Clone SMI 35 detects phosphorylated forms NF-M and NF-H, and may be used to detect early neuronal cell pathology and intraneuronal neurofibrillary tangles in Alzheimer's disease. Clone SMI 310, similar to clone SMI 31, recognizes phosphorylated NF-H, and demonstrates strong reaction with extraneuronal (ghost) neurofibrillary tangles in AD.

Neurofilament L/M/H Antibody Sampler Kit Contents:

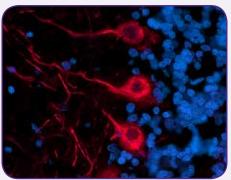
redionament E/M/11/Antibody Sampler Ait Contents.					
Specificity	Clone	Format	Reactivity	Application	
Neurofilament L (NF-L)	NFL3	Alexa Fluor® 647	Hu, Ms, Rat	IHC-P	
Neurofilament H (NF-H), Nonphosphorylated	SMI 32	Alexa Fluor® 488	Hu, Ms, Rat	IHC-P	
Neurofilament H (NF-H), Phosphorylated	SMI 31	Alexa Fluor® 594	Hu, Ms, Rat	IHC-P	
Neurofilament H & M (NF-H/NF-M), Hypophosphorylated	SMI 35	Alexa Fluor® 594	Hu, Ms, Rat	ICC	
Neurofilament H & M (NF-H/NF-M), Phosphorylated	SMI 310	Alexa Fluor® 488	Hu, Ms, Rat	IHC-P	

Neurofilament H & M, Phosphorylated



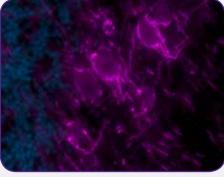
IHC staining of Alexa Fluor[®] 488 anti-Neurofilament H & M (NF-H/NF-M), Phosphorylated antibody (clone SMI 310, green) on FFPE human cerebellum tissue. Nuclei were counterstained with DAP (blue).

Neurofilament H Nonphosphorylated



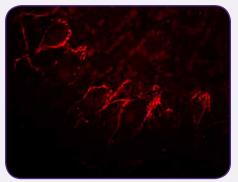
IHC staining of Alexa Fluor® 594 anti-Neurofilament H (NF-H), Nonphosphorylated antibody (clone SMI 32, red) on FFPE rat brain tissue. Nuclei were counterstained with DAPI (blue).

Neurofilament L



IHC staining of Alexa Fluor® 647 anti-Neurofilament L (NF-L) antibody (clone NFL3, magenta) on FFPE human brain tissue. Nuclei were counterstained with DAPI (blue).

Neurofilament H & M, Hypophosphorylated



IHC staining of Alexa Fluor* 594 anti-Neurofilament H & M (NF-H/NF-M), Hypophosphorylated (clone SMI 35) antibody on FFPE rat brain tissue.

Glial Cell Markers

Glial cells play an important role in the maintenance of normal nervous system physiology. Glial cells include microglia, astrocytes, and oligodendrocytes. Microglia are the resident phagocytes in the CNS, and actively survey their surrounding domain to guickly respond to an immune threat. As a consequence, these cells change shape to become amoeboidlike and become phagocytic to remove the encountered threat. Astrocytes are the most abundant glial cell type residing in the brain. They have many important functions, some of which include neurotransmitter uptake and release, and modulation of synaptic transmission. Astrocytes, like microglia, are highly sensitive to alterations in their microenvironment, and alter their morphology and gene expression profile to upregulate expression and secretion of a variety of bioactive molecules, such as cytokines and chemokines, in response to CNS injury. Oligodendrocytes produce myelin sheath to allow for the insulation of segments of neuronal axons. This enables high velocity signal transduction, which is essential for the propagation of action potentials along the axon. ODs also contribute to neuroplasticity and provide trophic support to neurons.

Glial Cell Marker Antibody Sampler Kit

The Glial Cell Marker Antibody Sampler Kit provides a great combination of antibodies to detect microglia, astrocytes, and oligodendrocytes. P2RY12 is a selective marker for microglia and does not stain other peripheral immune cell types. Similarly, CX3CR1 is a marker commonly used to detect microglia. However, this is also a common marker for other phagocytes such as macrophages. Usage of a combination of P2RY12 and CX3CR1 may allow the distinction between resident vs. peripheral immune cells. This distinction becomes important under inflammatory conditions where peripheral cells infiltrate the brain. GFAP is a gold standard marker not only for visualizing resting astrocytes, but is also widely used to detect reactive astrocytes, which stain much more strongly with GFAP antibodies than normal astrocytes. Reactive astrocytes are detectable under damage and many disease states resulting in astrogliosis. Myelin CNPase and myelin basic protein (MBP) are specific markers that allow identification of oligodendrocytes. These markers may be of particular interest to those who study ODs especially within a disease context, such as in multiple sclerosis, where autoreactive antibodies against MBP have been shown to contribute to the pathogenesis and destruction of the myelin sheaths.

Glial Cell Marker Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
P2RY12	S16007D	Purified	Ms	IHC-P, FC
CX3CR1	8E10.D9	Purified	Hu	IHC-P
GFAP	SMI 24	Purified	Hu, Ms, Rat	IHC-P, WB
Myelin CNPase	SMI 91	Purified	Hu, Ms, Rat	IHC-P, WB
Myelin Basic Protein	P82H9	Purified	Hu, Rat	IHC-P, WB

Learn more about microglia, astrocytes and oligodendrocytes at:

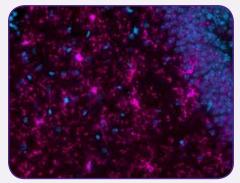
biolegend.com/microglia biolegend.com/astrocytes biolegend.com/oligodendrocytes

GFAP



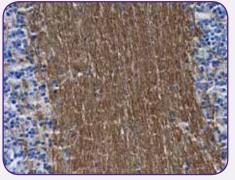
IHC staining of purified anti-GFAP antibody (clone SMI 24) on FFPE Rat brain tissue. The section was counterstained with hematoxylin.

P2RY12



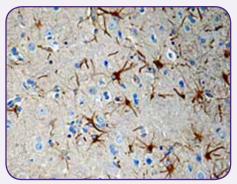
IHC staining of purified anti-P2RY12 antibody (clone S16007D, magenta) on FFPE mouse brain tissue. Nuclei were counterstained with DAPI (blue).

Myelin CNPase



IHC staining of purified anti-Myelin CNPase antibody (clone SMI 91) on FFPE rat brain tissue. The section was counterstained with hematoxylin.

CX3CR1



IHC staining of anti-CX3CR1 antibody (clone 8E10.D9) on FFPE normal human brain tissue. The section was counterstained with hematoxylin and bluing solution.

Synapse

A synapse, where an axon terminal (presynapse) meets a dendrite (postsynapse), is the functional unit of the brain, and the site of connection between neurons or neuro-glial cells. Structural and functional alterations to the presynaptic terminals, and synapse loss are a common feature of many neurodegenerative disorders and are associated with sensory, motor, and cognitive impairments. In order to study synapses and how they are affected in disease, highly specific antibodies are needed that allow visualization of these structures, assessment of protein subcellular localization, protein co-localization, and expression within the pre- and postsynaptic compartments by microscopy or biochemical assays namely WB.

Presynaptic Vesicle and Postsynaptic Antibody Sampler Kits

We offer a number antibodies for pre- and postsynaptic targets that are ideal for IHC, ICC, and WB. Our Presynaptic Vesicle Antibody Sampler Kit contains antibodies for proteins involved in glutamate transport (VGlut1, SVOP), synaptic vesicle membrane fusion, and neurotransmitter release (Syntaxin, Synapsin, SNAP-25). The Postsynaptic Antibody Sampler Kit includes antibodies for major scaffold proteins Shank, PSD95, and PSD93. This kit also contains antibodies for SAP102, a member of the membrane-associated guanylate kinase (MAGUK) superfamily as well as a pan-MAGUK reactive antibody.

Visit our synaptic function webpage or download our synaptic function information sheet to learn about additional products:

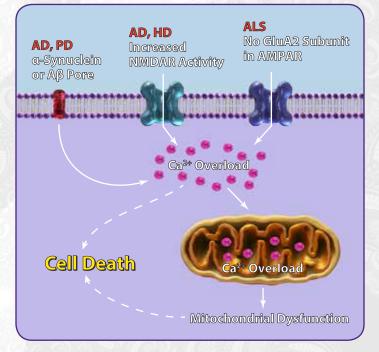
<u>biolegend.com/synaptic_function</u> <u>biolegend.com/literature</u>

Presynaptic Vesicle Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
VGlut1	N28/9	Purified	Hu, Ms, Rat	IHC-P, WB
Synapsin I/II/III	A17080A	Purified	Hu, Rat	IHC-P, WB
SVOP	N356/9	Purified	Hu, Ms, Rat	IHC-P, WB
Syntaxin	SP8	Purified	Hu, Ms, Rat	IHC-P, WB
SNAP-25	SMI 81	Purified	Hu, Ms, Rat	IHC-P, WB

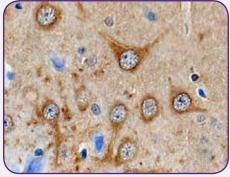
Postsynaptic Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
Pan-Shank	N23B/49	Purified	Hu, Ms, Rat	IHC-P, WB
MAGUK (pan reactive)	K28/86	Purified	Hu, Ms, Rat	IHC-P, WB
PSD95	K28/74	Purified	Hu, Ms, Rat	IHC-P, WB
PSD-93 (Chapsyn-110)	N18/28	Purified	Hu, Ms, Rat	IHC-P, WB
SAP102	N19/2	Purified	Hu, Ms, Rat	IHC-P, WB



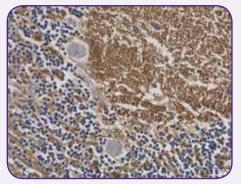
Perturbation of calcium homeostasis in neurodegenerative disorders. Glutamate-mediated excitotoxicity through sustained NMDAR and AMPAR activity affects cytosolic calcium levels in AD, ALS and Huntington's disease (HD). A β oligomers and mutant huntingtin protein were shown to increase NDMAR activity leading to the vulnerability of neurons to excitotoxicity. In ALS, neurons become vulnerable to glutamate toxicity mediated by AMPARs due to the absence of the GluA2 subunit, which interferes with the calcium permeability of AMPAR. Calcium overload and neurotoxicity can also be caused by the formation of calcium-permeable channels by a-Synuclein and A β oligomers. Mitochondria are important regulators of calcium, and take up and reduce cytosolic levels of this ion. However, high mitochondrial calcium levels can trigger depolarization, opening of the mitochondrial permeability transition pore, and the release of apoptotic factors leading to cell death.

SVOP



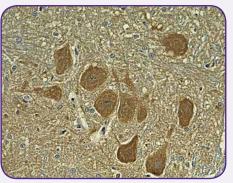
IHC staining of purified anti-SVOP antibody (clone N356/9) on FFPE rat brain tissue. The section was counterstained with hematoxylin.

SNAP-25



IHC staining of purified anti-SNAP-25 antibody (clone SMI 81) on FFPE human brain tissue. The section was counterstained with hematoxylin.

PSD95



IHC staining of purified anti-PSD95 antibody (clone K28/74) on FFPE rat brain tissue. The section was counterstained with hematoxylin.

Synaptic Receptors

Calcium signaling plays a fundamental role in normal brain physiology, and is essential for many processes including synaptic activity, cell-cell communications, activity-dependent synaptic remodeling, and memory formation. Activation of synaptic receptors such as NMDA receptors (NMDAR) result in the influx of calcium, activating signaling pathways that regulate various cellular functions. Calcium homeostasis must be tightly regulated due to its involvement in a multitude of pre- and post-synaptic processes. Increased excitatory stimulation and sustained calcium overload can lead to the dysregulation of cytosolic calcium homeostasis, and is detrimental for cellular health. Perturbations in cytosolic calcium levels have been observed in neurodegenerative diseases such as AD, PD and amyotrophic lateral sclerosis (ALS). In AD, deregulation of calcium levels has been linked to excitotoxicity mediated by AB-induced increase in NMDAR activity. In ALS, motor neurons become vulnerable to AMPA receptor (AMPAR)-mediated excitotoxicity in part due to the absence of the GluA2 subunit rendering AMPA receptors permeable to calcium³.

GluA1/2 and NMDA (GluN) Receptor Antibody Sampler Kits

To complement our Presynaptic Vesicle and Postsynaptic Antibody Sampler kits, we offer sampler kits for AMPA and NMDA receptors. The GluA1/2 Receptor Antibody Sampler Kit allows detection of AMPAR subunits GluA1 and GluA2, and proteins involved in the regulation of AMPA receptor content: PICK1 and SynDYG1. PSD95 was included as a control for postsynapse identification. The NMDA (GluN) Receptor Antibody Sampler Kit contains antibodies specific for NMDA receptor subunits GluN1, GluN2A, and GluN2B. The kit also includes antibodies for PSD95 and Shank to allow protein localization assessment.

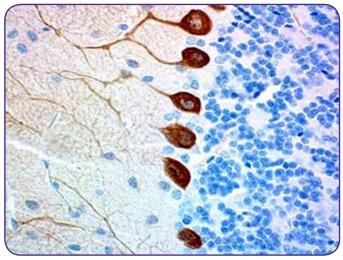
GluA1/2 Receptor Antibody Sampler Kit Contents:

		/ /		
Specificity	Clone	Format	Reactivity	Application
GluR1	N355/1	Purified	Ms, Rat	IHC-P, WB
GluR2	L21/32	Purified	Hu, Ms, Rat	IHC-P, WB
PICK1	L20/8	Purified	Hu, Ms, Rat	IHC-P, WB
PSD95	K28/74	Purified	Hu, Ms, Rat	IHC-P, WB
SynDIG1	L42/17	Purified	Hu, Ms, Rat	WB

NMDA (GluN) Receptor Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application
NMDAR1	N308/48	Purified	Ms, Rat	IHC-P, WB
NMDAR2A	N327/95	Purified	Ms, Rat	WB
NMDAR2B	N59/36	Purified	Hu, Ms, Rat	IHC-P, WB
PSD95	K28/74	Purified	Ms, Rat	IHC-P, WB
Pan-Shank	N23B/49	Purified	Hu, Ms, Rat	IHC-P, WB

Pan-Shank



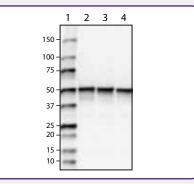
IHC staining of purified anti-Pan-Shank antibody (clone N23B/49) on FFPE rat cerebellum tissue. The section was counterstained with hematoxylin.

GluR2



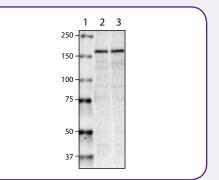
IHC staining of purified anti-GluR2 antibody (clone L21/32) on FFPE rat brain tissue. The section was counterstained with hematoxylin.

PICK1

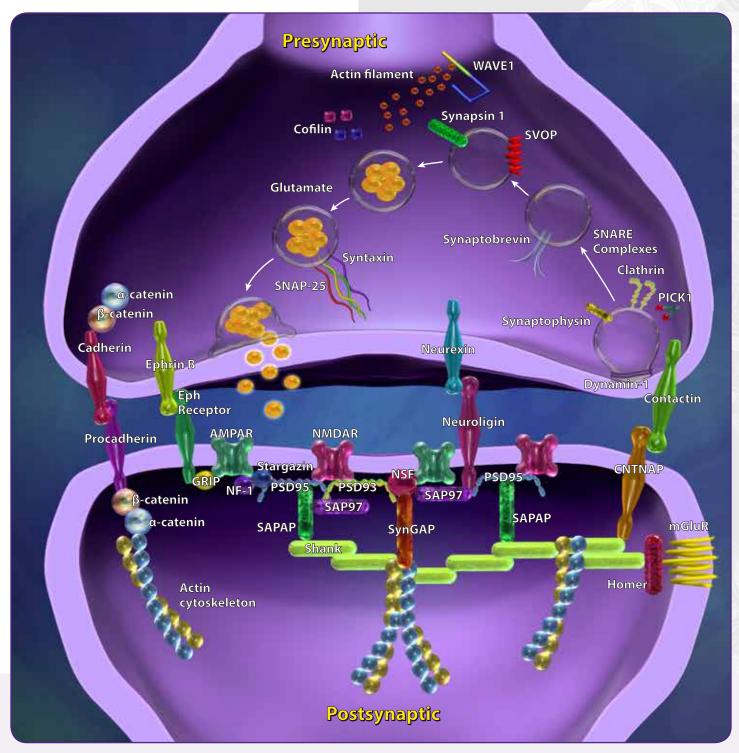


Western blot of purified anti-PICK1 antibody (clone L20/8). Lane 1: Molecular weight marker; Lane 2: 20 µg of human brain membrane lysate; Lane 3: 20 µg of mouse brain membrane lysate; Lane 4: 20 µg of rat brain membrane lysate.

NMDAR2A



Western blot of purified anti-NMDAR2A antibody (clone N327/95). Lane 1: Molecular weight marker; Lane 2: 20 µg of mouse brain membrane lysate; Lane 3: 20 µg of rat brain membrane lysate.



Schematic representation of pre- and postsynaptic compartments in an excitatory neuronal synapse. The excitatory neurotransmitter glutamate is stored and released from the presynaptic vesicles at the presynaptic terminal. Glutamate binds to and activates AMPA, NMDA, and metabotropic receptors clustered at the postsynaptic membrane within small membrane protrusions known as dendritic spines. These receptors in turn are connected to scaffolding proteins (*e.g.* PSD95 and Shank), which in turn can recruit signaling complexes, such as protein kinases and phosphatases. Actin filaments provide structural support and help shape dendritic spines. They can also indirectly link various components with these spines to allow the regulation of spine development and morphology.

Autophagy

Autophagy is one of the major protein degradation machineries responsible for the breakdown and turnover of abnormal proteins and damaged organelles to maintain cellular homeostasis. Autophagy plays an important role in degradation of key misfolded proteins involved in the pathogenesis of neurodegenerative disorders. Dysfunction in the autophagy pathway impedes the clearance of aggregate-prone targets, and has been associated with accelerated disease progression. Furthermore, impairment in the maturation of autophagosomes to autolysosomes, resulting in the accumulation of autophagic vacuoles and autophagy-related vesicular compartments, has been observed in neurodegenerative disorders.

Autophagy Antibody Sampler Kit

The Autophagy Antibody Sampler Kit offers antibodies for targets known as protein modifiers (e.g. ubiquitin), autophagy initiators (e.g. beclin-1), and proteins responsible for extension and maturation of autophagosomes (e.g. ATG5, LC3, and Rab7A). The components of this kit are each essential for evaluating autophagy or protein modification under normal and pathological conditions. Anti-ubiquitin antibodies are commonly used in immunohistochemistry to detect proteinaceous inclusions that are formed in a variety of neurodegenerative diseases, as ubiguitinated species of misfolded proteins are the major constituents of these inclusions and often accumulate in the cytosol. For instance, neurofibrillary tangles and dystrophic neurites containing Tau inclusions can be visualized by ubiquitin as well as Tau immunoreactivity. Furthermore, proteins destined for degradation by autophagy can be conjugated to ubiquitin. This conjugation serves as a signal for the recruitment and binding of adaptor proteins such as p62, which in turn can bind to LC3, and lead to the clearance of ubiquitin-conjugated proteins.

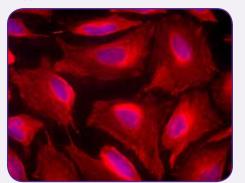
Beclin-1 is part of the PI3K complex that facilitates the generation of phosphatidylinositol 3-phosphate and the formation of phagophores. Antibodies against beclin-1 are used to detect its subcellular localization and expression. Anti-AGT5 antibodies are utilized as a marker for newly formed phagophores, also referred to as isolation membranes. LC3 is initially cleaved by ATG4 to generate LC3-I, which is subsequently converted to LC3-II as a result of conjugation to phosphatidylethanolamine by the ATG5 complex. LC3-II remains associated with autophagosomes and hence is the most common marker for detection of these structures.

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Specificity	Clone	Format	Reactivity	Application			
Ubiquitin	P4G7	Purified	Extensive (Yeast to Hu)	IHC-P, WB			
Rab7A	W16034A	Purified	Hu, Ms, Rat	IHC-P, ICC, WB, Direct ELISA			
Beclin-1	O93F3	Purified	Hu, Ms, Rat	WB			
ATG5	177.19	Purified	Hu, Ms, Rat	IHC-P, ICC, WB			
LC3	A15143K	Purified	Ms, Ms	IHC-P, ICC			

Autophagy Antibody Sampler Kit Contents:

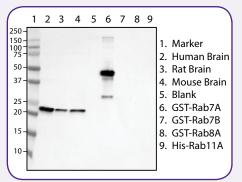
To learn more about autophagy, download our poster at: biolegend.com/literature

ATG5



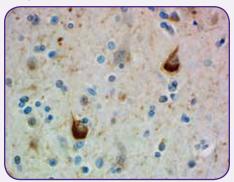
ICC staining of purified anti-ATG5 antibody (clone 177.19, red) on HeLa cells. Nuclei were counterstained with DAPI (blue).

Rab7A



Western blot of purified anti-Rab7A antibody (clone W16034A). M: Molecular weight marker; brain lysates: 20 µg; recombinant proteins: 10 ng.

Ubiquitin



IHC staining of purified anti-Ubiquitin antibody (clone P4G7) on FFPE AD brain tissue. The section was counterstained with hematoxylin.

Epitope Tag Antibody Sampler Kits

The Epitope Tag Small and Big Motif Antibody Sampler Kits provide a range of antibodies to conveniently detect proteins fused to small and large epitope tags, respectively, by a number of applications including WB, IHC, and ICC.

Epitope Tag Small Motif Antibody Sampler Kit Contents:

Specificity	Clone	Format	Reactivity	Application		
Anti-DYKDDDDK	L5	Purified	DYKDDDDK Tag Epitope	WB, IP, IF, IHC, ELISA, FC, Purification		
Anti-c-Myc	9E10	Purified	EQKLISEEDL Tag Epitope	WB, IP, IF, IHC, ELISA, Purification		
Anti-HA.11	16B12	Purified	YPYDVPDYA Tag Epitope	WB, IF, IP, Purification		
Anti-V5 Tag	7/4	Purified	GKPIPNPLLGLDST Tag Epitope	WB, IP		
Anti-His Tag	J099B12	Purified	6x His Tag Epitope	WB, ICC, ICFC		

Epitope Tag Big Motif Antibody Sampler Kit Contents:

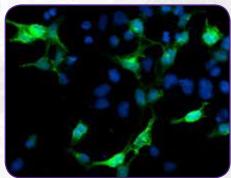
Specificity	Clone	Format	Reactivity	Application		
Anti-GST	P1A12	Purified	GST	WB, Purification		
Anti-GFP	1GFP63	Purified	GFP	WB		
Anti-mCherry	8C5.5	Purified	mCherry	WB, IF, IP		
Anti-Thioredoxin 1	4H12A59	Purified	<i>E. coli</i> Thioredoxin-1	WB, IP		

Download our information sheet on epitope tag antibodies to learn more about our products at: <u>biolegend.com/literature</u>

References

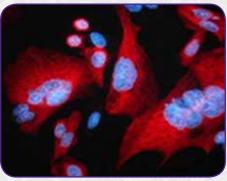
- 1. Ouelati A, et al. 2010. Progress in Brain Research. 183:115
- 2. Yyan A, et al. 2017. Cold Spring Harb Perspect Biol. 9(4):1
- 3. Marambaud, et al. 2009. Mol Neurodegener. 4:20

mCherry



mCherry-transfected 293T cells were stained with purified anti-mCherry (clone 8C5.5) antibody, followed by staining with DyLight[™] 488 Goat anti-mouse IgG antibody (green). Nuclei were counterstained with DAPI (blue).

HA.11

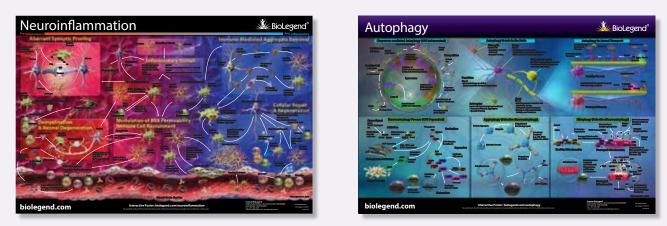


Staining of Clone 16B12 on methanol fixed CHO cells transfected with an HA-tagged protein at 1 μ g/ml. Cells were then incubated with Alexa Fluor[®] 594 Goat anti-mouse IgG secondary and were counterstained with DAPI (blue).

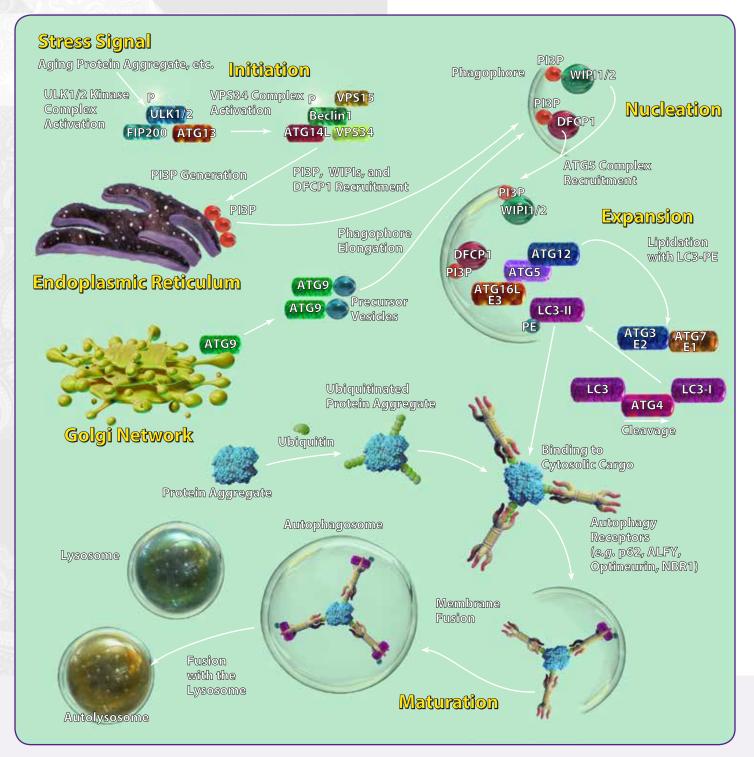
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Schematic representation of ATG-dependent macroautophagy and aggrephagy pathways. Macroautophagy can be induced by a variety of stress signals including protein aggregates. The process of autophagosome formation and fusion with the lysosome is divided into four steps: 1) initiation, 2) nucleation, 3) expansion, and 4) maturation. In the initiation phase, the ULK1/2 kinase complex is activated by phosphorylation of ULK1/2, which in turn activates the VPS34 complex, formed through its association with Beclin-1, VPS15, and Atg14. This complex drives the generation of PI3P on the ER. PI3P's interactions with WIPIs and DECP1, together with ATG9 localized to TGN-derived precursor vesicles, drive the nucleation, and biogenesis of the phagophore. The expansion phase involves ATG4-mediated cleavage of LC3-I, and the recruitment of the ATG5 complex to the phagophore to stimulate the conjugation of LC3-I to phosphatidylethanolamine (PE) to generate LC3-II-PE. Aggrephagy is a term used for the selective degradation of protein aggregates. Ubiquitination of these aggregates signals the recruitment and binding of autophagy receptors, such as p62, to the aggregates and their delivery to the phagophore through binding to LC3-II-P. In the maturation phase, the autophagosomes fuse with the lysosomes to form autolysosomes. Acidification of this compartment leads to degradation of cytosolic protein sincluding protein aggregates.

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Customer Service:

US & Canada Toll-Free: 1.877.246.5343 (877-BIOLEGEND) International: 1.858.768.5800 Fax: 1.877.455.9587 *email: cs@biolegend.com*

Technical Service:

US & Canada Toll-Free: 1.877.273.3103 International: 1.858.768.5801 email: tech@biolegend.com

Headquarters:

BioLegend 8999 BioLegend Way San Diego, CA 92121 USA

International Offices

Europe:

BioLegend 4B Highgate Business Centre 33 Greenwood Place London NW5 1LB United Kingdom Tel: +44 (0) 20 3475 3880 Fax: +44 (0) 20 3318 3271 email Inquiries: infoeurope@biolegend.com email Technical Support: techeurope@biolegend.com

Japan:

BioLegend 8F, SB bldg., 1-4-6, Nezu, Bunkyo-ku, Tokyo 113-0031, Japan Tel: +81-3-3823-9071 Fax: +81-3-3823-9072 email: supportjp@biolegend.com biolegend.com/jp

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