

NEUROSCIENCE FOCUS: NEUROTROPHIC FACTORS

VPS10P-domain Receptor Family

FEATURED DATA:

APP · BDNF · β -NGF · NGF R · Phospho-APP · Phospho-TrkA · Phospho-TrkB · Phospho-TrkC · SorCS2 · SorLA · Sortilin · TrkA

VPS10P-domain Receptors

Vacuolar protein sorting 10 protein (VPS10P)-domain receptors are type I transmembrane proteins that bind a range of ligands including neurotrophins, neuropeptides, and other transmembrane proteins. Additional studies suggest novel roles for VPS10P-domain receptors in ciliary neurotrophic factor (CNTF) signaling, and in the trafficking of Lipoprotein Lipase (LPL) and Cholesterol. In vertebrates, there are five members of the family, Sortilin, sorting protein-related receptor with A-type repeats (SorLA), Sortilin-related receptor CNS expressed 1 (SorCS1), SorCS2, and SorCS3. These multifunctional molecules have been shown to affect neuronal viability and function by regulating protein transport and signal transduction. Each receptor is expressed in distinct neuronal populations, suggesting discrete functions in different cell types.

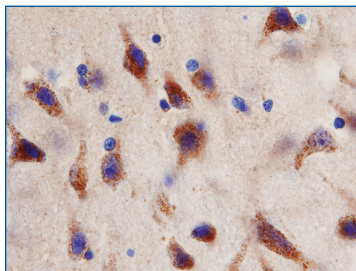
Variable function between family members is supported by the subcellular expression of each receptor. Sortilin and SorLA are predominantly found intracellularly, in the trans-Golgi network (TGN), with less than 10% at the cell surface. Subcellular trafficking of SorCS1 is dependent on the splice variant. SorCS1a is predominantly intracellular, SorCS1b is expressed at the cell surface, and SorCS1c is evenly divided between the two. SorCS2 and SorCS3 are also cell surface receptors but do not appear to direct major intracellular trafficking. In all family members, the VPS10P domain is believed to adopt a β -propeller fold structure and functions as the protein interaction motif.

R&D Systems Products for VPS10P-domain Receptors

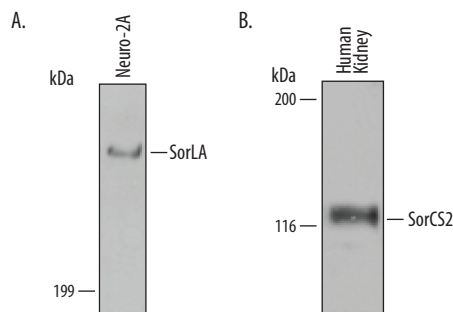
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES
SorCS1	H M	H (IHC, WB)
SorCS2	H M	H (WB) M (IHC, WB)
SorCS3	H M	H (WB) M (B/N, IHC, WB)
SorLA		H (IHC, WB) M (IHC, WB)
Sortilin	H M	H (B/N, IHC, WB) M (B/N, IHC, WB)

Species Key: H Human M Mouse Application Key: B/N Blocking/Neutralization IHC Immunohistochemistry WB Western blot

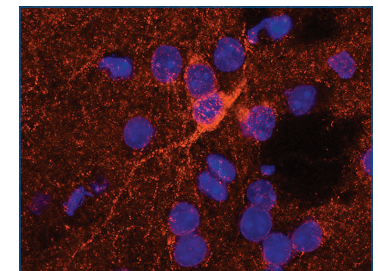
For more information visit our website at
www.RnDSystems.com/go/VPS10P



SorLA in Human Cortex. Sorting protein-related receptor with A-type repeats (SorLA) was detected in immersion-fixed paraffin-embedded sections of human brain using a Mouse Anti-Human SorLA Monoclonal Antibody (Catalog # MAB5699). Before incubation with the primary antibody, the tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). The tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS002; brown) and counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasmic granules.



Detection of Mouse SorLA and Human SorCS2 by Western Blot. Western blots show lysates of the Neuro-2A mouse neuroblastoma cell line and human kidney (medulla) tissue. A. The PVDF Membrane was probed with a Sheep Anti-Human/Mouse SorLA Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5699) followed by a HRP-conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). B. The PVDF Membrane was probed with a Sheep Anti-Human SorCS2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4238) followed by a HRP-conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). SorLA and SorCS2 were detected at approximately 250 kDa and 120 kDa, respectively (as indicated).



SorCS2 in Mouse Piriform Cortex. Sortilin-related receptor CNS expressed 2 (SorCS2) was detected in immersion-fixed frozen sections of mouse brain using a Sheep Anti-Mouse SorCS2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4237). The tissue was stained using the NorthernLights™ 557-conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # NL010; red) and counterstained with DAPI (blue). Specific labeling was localized to neuronal cell bodies and processes.

Modulation of Neurotrophin Signaling

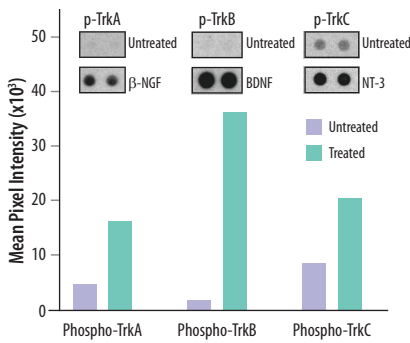
Neuroscience-related research of VPS10P-domain receptors has focused on two main areas, modulation of neurotrophin signaling and amyloid precursor protein (APP) cleavage. The neurotrophin family includes β -Nerve Growth Factor (NGF), Brain-derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin-4 (NT-4). In addition, pro-neurotrophin precursors are known to be secreted functional molecules that induce distinct signaling pathways, compared to their corresponding cleaved mature form. In general, mature neurotrophins exert their biological effects on neuronal viability through tropomyosin kinase (Trk) receptors. Trk signaling is known to be potentiated by Trk-dependent cleavage of the intracellular domain of neurotrophin receptor p75 (NGF R). In contrast, pro-neurotrophins induce apoptosis in neurons via NGF R, an action that is not dependent on Trk receptors but is dependent on interactions between both the pro-neurotrophin domain and Sortilin, and the mature domain and NGF R. In addition to affecting ligand binding, Sortillin may also modulate neurotrophin signaling by regulating intramembrane proteolysis of NGF R.

R&D Systems Products for Neurotrophin Family Ligands & Receptors

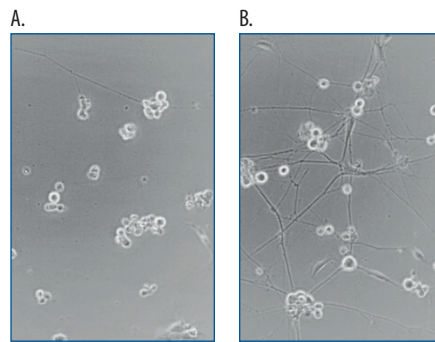
MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
Pro-BDNF		H (IHC, WB)	
BDNF	H	H (ELISA, FC, IHC, WB)	H
LINGO-1		H (FC, IHC, WB)	
β -NGF	H M R	H (B/N, ELISA, IHC, WB) R (B/N, ELISA, IHC, WB)	H R
NGF R/TNFRSF16	H M	H (FC, IHC, WB) M (IHC, WB)	
NT-3 Propeptide		H (WB)	

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
NT-3	H	H (B/N, ELISA, IHC, WB)	H
Sortilin	H M	H (B/N, IHC, WB) M (B/N, IHC, WB)	
TrkA	H R	H (B/N, FC, IHC, WB) R (IHC, WB)	H R
TrkB	H M	H (FC, IHC, WB) M (B/N, IHC, WB)	H
TrkC	H M	H (B/N, FC, IHC, WB) M (B/N, IHC, WB)	H
TROY/TNFRSF19	H M	H (WB) M (ELISA, IHC, WB)	M

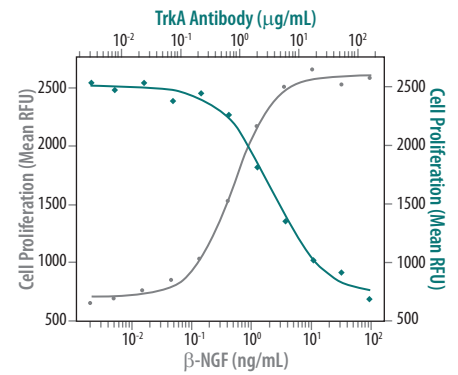
Species Key: H Human M Mouse R Rat Application Key: B/N Blocking/Neutralization ELISA ELISA Capture and/or Detection FC Flow Cytometry IHC Immunohistochemistry WB Western blot



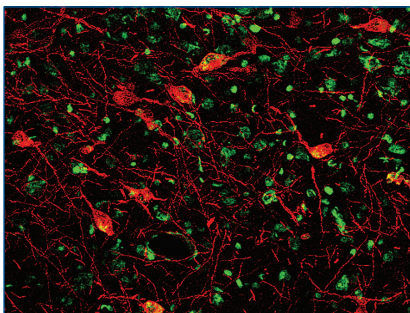
Detection of Neurotrophin-induced Receptor Tyrosine Kinase Phosphorylation using the Proteome Profiler™ Human Phospho-RTK Array. The C6 rat glial cell line was transfected with human TrkA, TrkB, or TrkC. Cells were untreated, or treated with Recombinant Human β -NGF (Catalog # 256-GF), Recombinant Human BDNF (Catalog # 248-BD), or Recombinant Human NT-3 (Catalog # 267-N3) for 5 minutes. Cell lysates were assessed for the relative levels of phosphorylation of TrkA, TrkB, TrkC, and thirty-nine other receptor tyrosine kinases (RTKs) using the Proteome Profiler Human Phospho-RTK Array Kit (Catalog # ARY001). Representative images of the pre-spotted nitrocellulose membranes are shown following chemiluminescent detection (inset).



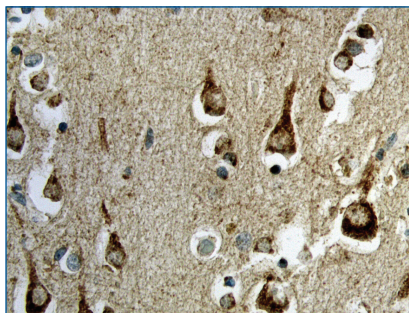
β -NGF Enhances Neurite Outgrowth. Chick dorsal root ganglion neurons (E10-11) were cultured for 3-days under control conditions (A) or in the presence of 16 ng/ml Recombinant Human β -NGF (B, Catalog # 256-GF). The presence of β -NGF significantly enhanced neurite outgrowth.



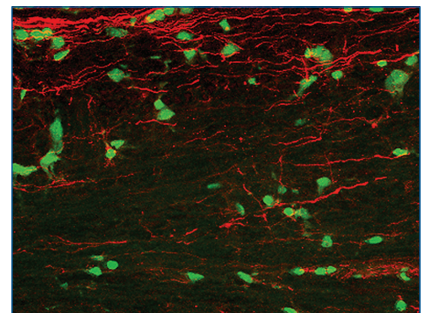
Cell Proliferation Induced by β -NGF and Neutralization by a TrkA Antibody. Recombinant Human β -NGF (Catalog # 256-GF) stimulates proliferation of the TF-1 human erythroleukemic cell line in a dose-dependent manner (green line). Proliferation elicited by 5 ng/ml Recombinant Human β -NGF is neutralized (blue line) by increasing concentrations of a Goat Anti-Human TrkA Antigen Affinity-purified Polyclonal Antibody (Catalog # AF175).



NGF R/TNFRSF16 in Mouse Striatum. Nerve Growth Factor Receptor (NGF R)/TNFRSF16 was detected in perfusion-fixed frozen sections of mouse brain using a Goat Anti-Mouse NGF R/TNFRSF16 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1157). The tissue was stained (red) and counterstained (green). Specific labeling was localized to neuronal cell bodies and processes.



Sortilin in Human Cingulate Cortex. Sortilin was detected in immersion-fixed paraffin-embedded sections of human brain using a Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154). The tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue). Specific labeling was localized to neuronal cell bodies and processes.



BDNF in Rat Dorsal Root Ganglion. Brain-derived Neurotrophic Factor (BDNF) was detected in frozen sections of rat spinal cord using a Chicken Anti-Human BDNF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF248). The tissue was stained (red) counterstained (green).

Modulation of APP Processing

Dysregulated protein trafficking by VPS10P-domain receptors has been implicated in various disease conditions, including Alzheimer's disease (AD) and other dementias, type 2 diabetes, and coronary heart disease. A pathological hallmark of AD is increased generation of amyloid- β protein (A β) and its deposition in the brain as senile plaques. Many groups have reported an inverse correlation between the expression levels of SorLA and A β in cultured neurons, and in the brains of transgenic mice overexpressing SorLA. Recent studies suggest the underlying mechanism may be dependent on a loss of SorLA-dependent trafficking of the amyloid precursor protein (APP).

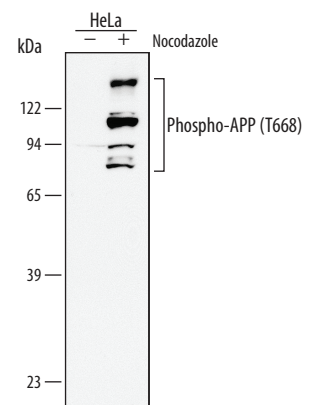
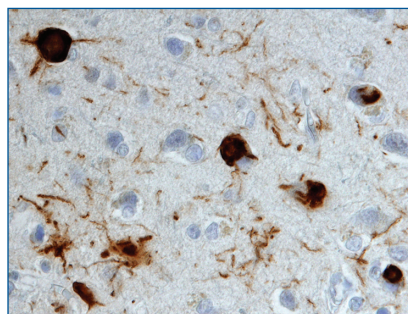
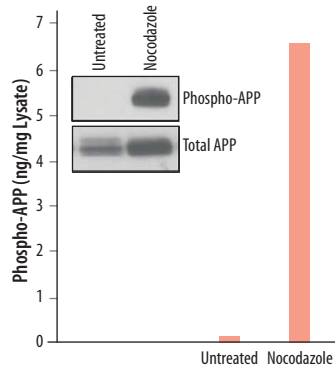
R&D Systems Products for APP Cleavage & A β Degradation

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
ACE	H M R	H (FC, IHC, IP, WB) M (ELISA, FC, IHC, IP, WB)	H M
Acetylcholinesterase/ACHE	M		
ADAM9	H M	H (ELISA, FC, IP, WB) M (FC, IHC, IP, WB)	H
ADAM10	H M	H (FC, IHC, IP, WB) M (FC, IHC, WB)	
ADAMTS4	H	H (B/N, IP, WB)	
APBA3		H (WB)	
APLP-1		H (FC, IHC, IP, WB) M (IP, WB)	
APLP-2		H (WB) M (WB)	
Apolipoprotein E/ApoE		H (WB)	
APP/Protease Nexin II	H	H (IHC, IP, WB) M (IHC, WB) R (WB) Ca (WB) Ch (WB) Pr (WB) Ms (WB)	H M R
APP 695+1		H (IHC, IP, WB)	
APP+1	H	H (WB)	
Arylsulfatase A/ARSA	H M	H (IHC, IP, WB)	
BACE-1	H M	H (B/N, FC, IHC, IP, WB) M (B/N, FC, IHC, IP, WB)	
BACE-2	M	H (IHC, IP, WB) M (IP, WB)	
Cathepsin B	H M	H (ELISA, IHC, IP, WB) M (B/N, IHC, WB)	H
Cathepsin D	H M	H (IHC, IP, WB) M (IHC, IP, WB)	
Cathepsin E	H M	H (IHC, IP, WB) M (IHC, IP, WB)	
Complement Component C1qC		H (IHC, WB)	
Cystatin C	H M R	H (B/N, ELISA, IHC, IP, WB) M (ELISA, IHC, IP, WB) R (IHC, IP, WB)	H M
DISC1		H (IHC, WB)	
DYRK1A		H (IHC, WB) R (IHC, WB)	
DYRK2		H (IHC, WB) M (WB) R (IHC, WB)	

MOLECULE	RECOMBINANT & NATURAL PROTEINS	ANTIBODIES	ELISAs
ECE-1	H M	H (FC, IHC, IP, WB)	
ECE-2	H	H (IHC, IP, WB)	
FPRL1		H (FC)	
F-Spondin/SPON1	H	H (B/N, WB)	
Glutamyl-peptide Cyclotransferase/QPCT	H		
Insulysin/IDE	H	H (FC, IP, WB)	
ITM2C		H (WB)	
Kallikrein 6/Neurosin	H	H (IHC, IP, WB)	
LRRTM3	H M	H (IHC, WB) M (IHC, WB)	
MMP-2	H M R	H (FC, IHC, IP, WB) M (IHC, IP, WB) R (IHC, IP, WB)	H
MMP-9	H M R	H (ELISA, FC, IHC, IP, WB) M (ELISA, IHC, IP, WB)	H M
Neprilysin/CD10	H M	H (ELISA, FC, IHC, IP, WB) M (B/N, ELISA, IHC, IP, WB)	H M
Neurolysin	H	H (IP, WB) M (IP, WB) R (IP, WB)	
Nicastrin		H (IHC, WB)	
Plasminogen	H	H (B/N, IP, WB)	
Presenilin-1		H (ELISA, IHC, WB)	H
Presenilin-2		H (IHC, WB)	
Progranulin	H M	H (IHC, IP, WB) M (ELISA, IHC, WB)	H M
Serpin A3/cx1-Antichymotrypsin	H	H (IP, WB)	
Serpin A3N	M	M (IP, WB)	
Serpin E2/PN1	H M	H (WB) M (B/N, IP, WB)	
TACE/ADAM17	H M	H (ELISA, FC, IHC, IP, WB)	H
Thimet Oligopeptidase/THOP1	H	H (IHC, IP, WB)	

Species Key: H Human M Mouse R Rat Ca Canine Ch Chicken Pr Primate Ms Multispecies

Application Key: B/N Blocking/Neutralization ELISA ELISA Capture and/or Detection FC Flow Cytometry IHC Immunohistochemistry IP Immunoprecipitation WB Western blot



Measurement of Phospho-APP (T668) Levels using the DuoSet[®] IC ELISA. Lysates prepared from the rat C6 glioma cell line which was either untreated or treated with nocodazole, were quantified using the Human/Mouse/Rat Phospho-APP (T668) DuoSet IC ELISA (Catalog # DY2508; bar graph). The same lysates were also immunoblotted (inset) using either a Rabbit Anti-Human/Mouse Phospho-APP (T668) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2508) or a Goat Anti-Human APP Pan Specific Affinity Purified Polyclonal Antibody (Catalog # AF1168).

Phospho-APP (T668) in Alzheimer's Disease Cortex. APP/Protease Nexin II was detected in immersion-fixed paraffin-embedded sections of human Alzheimer's disease brain using a Rabbit Anti-Human/Mouse Phospho-APP (T668) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2508). The tissue was stained with the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS005; brown) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm, plasma membrane, and processes of cortical neurons.

Detection of Human Phospho-APP (T668) by Western Blot. Western blot shows lysates of the HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with nocodazole. The PVDF membrane was probed with a Mouse Anti-Human/Mouse Phospho-APP (T668) Monoclonal Antibody (Catalog # MAB2508), followed by a HRP-conjugated Goat Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). Multiple bands represent alternative splicing and cleavage of APP.



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FC114_VPS10P_SEP

Recent Citations: R&D Systems References for VPS10P-Domain Receptor Family

- Vaegter, C. *et al.* (2011) Sortilin associates with Trk receptors to enhance anterograde transport and neurotrophin signaling. *Nat. Neurosci.* 14:54.

Recombinant Mouse NGF R/TNFRSF16 Fc Chimera (Catalog # 1157-NR)

Recombinant Mouse Ret Fc Chimera (Catalog # 482-RT)

Recombinant Human TrkA Fc Chimera (Catalog # 175-TK)

Recombinant Human TrkB Fc Chimera (Catalog # 688-TK)

Recombinant Human TrkC Fc Chimera (Catalog # 373-TC)

Sample: Soluble Sortilin
Application: Surface plasmon resonance

Goat Anti-Mouse Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2934)

Goat Anti-Mouse TrkC Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1404)

Sample: Mouse superior cervical and dorsal root ganglion
Application: Immunofluorescence

Goat Anti-Mouse Ret Antigen Affinity-purified Polyclonal Antibody (Catalog # AF482)

Goat Anti-Mouse TrkB Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1494)

Sample: Mouse hippocampal and cortical neuron immunoprecipitates
Application: Western blot
- Musunuru, K. *et al.* (2010) From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. *Nature* 466:714.

Goat Anti-Mouse Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2934)

Sample: Human liver homogenate
Application: Western blot
- Hu, F. *et al.* (2010) Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. *Neuron* 68:654.

Goat Anti-Mouse Sortilin Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF2934)

Sample: Mouse brain homogenate and serum
Application: Western blot

Sheep Anti-Mouse Progranulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2557)

Sample: Mouse brain homogenate and serum, and mouse brain
Application: Western blot and immunofluorescence (respectively)

Mouse Anti-Human Progranulin Monoclonal Antibody (Clone 296628) (Catalog # MAB2420)

Sample: HEK293 human embryonic kidney cell line transfected with human progranulin and C13-NJ human microglial cells
Application: Western blot and immunofluorescence (respectively)
- Reitz, C. *et al.* (2011) SORCS1 alters amyloid precursor protein processing and variants may increase Alzheimer's disease risk. *Ann. Neurol.* 69:47.

Goat Anti-Human SorCS1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3457)

Sample: HEK293 human embryonic kidney cell line transfected with human SorCS1, and SH-SY5Y human neuroblastoma cell line
Application: Western blot and immunofluorescence (respectively)
- Ahmed, R. *et al.* (2010) BACE1 and BACE2 enzymatic activities in Alzheimer's disease. *J. Neurochem.* 112:1045.

Mouse Anti-Human/Mouse BACE-1 Monoclonal Antibody (Clone 137612) (Catalog # MAB931)

Sheep Anti-Human BACE-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4097)

Sample: Human brain homogenate
Application: Immunoprecipitation and Western blot

Goat Anti-Human/Mouse BACE-1 ECD Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF931)

Sheep Anti-Human BACE-2 Biotinylated Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF4097)

Sample: Human brain homogenate
Application: ELISA development

Recombinant Human BACE-1 (Catalog # 931-AS)

Recombinant Mouse BACE-2 (Catalog # 2977-AS)

Recombinant Human Cathepsin D (Catalog # 1014-AS)

Sample: Recombinant Human APP/Protease Nexin II
Application: Enzymatic cleavage assay

Recombinant Human APP/Protease Nexin II (Catalog # 3466-PI)

Sample: Substrate for recombinant BACE-1, BACE-2 and Cathepsin D
Application: Enzymatic cleavage assay

For more information visit our website at
www.RnDSystems.com/go/VPS10P