#5887 Store at -20°C

γ Secretase Antibody Sampler Kit

☑ 1 Kit
(4 x 20 µl)

Cell Signaling TECHNOLOGY®

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rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Nicastrin (D38F9) Rabbit mAb	5665	20 µl	110, 120 kDa	Rabbit IgG
PEN2 (D6G8) Rabbit mAb	8598	20 µl	13 kDa	Rabbit IgG
Presenilin 1 (D39D1) Rabbit mAb	5643	20 µl	22 kDa	Rabbit IgG
Presenilin 2 (D30G3) Rabbit mAb	9979	20 µl	23 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The γ Secretase Antibody Sampler Kit provides an economical means of evaluating components of the gamma secretase complex. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The γ secretase protease complex interacts with and cleaves intramembrane substrates as an essential function for regulation of intracellular signaling and cell-cell interactions. This multiprotein complex is comprised of four integral membrane proteins, Presenilin, Nicastrin, Aph-1, and PEN2, all of which are essential for complete proteolytic activity (1). Presenilin 1 and presenilin 2 are transmembrane proteins belonging to the presenilin family. Mutation of presenilin genes has been linked to early onset of Alzheimer disease, probably due to presenilin's associated γ-secretase activity for amyloid-β protein processing (2,3). Endogenous presenilin mainly exists in a heterodimeric complex formed from the endoproteolytically processed amino-terminal (34 kDa) and carboxy-terminal (~20, 22, 23 kDa) fragments (CTF) (3, 4). Nicastrin is a transmembrane glycoprotein serving as an essential component of the γ-secretase complex (5,6). Nicastrin protein is physically associated with presenilin and plays an important role in stabilization and correct localization of presenilin to the membrane-bound γ-secretase complex (7). Nicastrin also serves as a docking site for γ-secretase substrates such as APP and Notch, directly binding to them and presenting them properly to γ-secretase to ensure the correct cleavage process (6,8). Presenilin Enhancer 2 (PEN2) is a small integral membrane glycoprotein that contains two recognized transmembrane domains. Both the N- and C-terminal domains are oriented into the lumen of the endoplasmic reticulum (9). It was found that PEN2 is an important part of the γ-secretase complex, and knocking it down results in reduced amounts of the complex, resulting in a loss of γ-secretase activity (10).

Specificity/Sensitivity: Nicastrin (D38F9) Rabbit mAb, PEN2 (D6G8) Rabbit mAb, Presenilin 1 (D39D1) Rabbit mAb, and Presenilin 2 (D30G3) Rabbit mAb recognize endogenous levels of respective target proteins. Presenilin 1 (D39D1) Rabbit mAb may also detect a nonspecific band at 60 kDa.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a recombinant protein specific to the carboxy terminus of human Presenilin 1 protein, a synthetic peptide corresponding to residues surrounding Val390 of human Nicastrin protein, a synthetic peptide corresponding to residues surrounding Leu92 of human PEN2 protein, or a synthetic peptide corresponding to residues surrounding Met323 of human Presenilin 2 protein.

Background References:

(1) Hansson, C.A. et al. (2004) *J Biol Chem* 279, 51654-60.
(2) Haass, C. and DeStrooper, B. (1999) Science 286, 916-919.
(3) Kimberly, W.T. et al. (2000) *J Biol Chem* 275, 3173-8.
(4) Kim, T.W. et al. (1997) *J Biol Chem* 272, 11006-10.
(5) Yu, G. et al. (2000) *Nature* 407, 48-54.
(6) Esler, W.P. et al. (2002) *Proc Natl Acad Sci U S A* 99, 2720-5.
(7) Kopan, R. and Goate, A. (2002) *Neuron* 33, 321-4.
(8) Chen, F. et al. (2001) *Nat Cell Biol* 3, 751-4.
(9) Sala Frigerio, C. et al. (2005) *J Neurol* 252, 1033-6.
(10) Steiner, H. et al. (2002) *J Biol Chem* 277, 39062-5.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#5887

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.