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Alzheimer’s Disease Antibody Sampler Kit

- 1 Kit (8 x 20 µl)

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

Description: The Alzheimer’s Disease Antibody Sampler Kit provides an economical means of evaluating Alzheimer’s Disease-related signaling. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Alzheimer’s Disease (AD) is one of the most common neurodegenerative diseases worldwide. Clinically, it is characterized by the presence of extracellular amyloid plaques and intracellular neurofilament tangles, which result in neuronal dysfunction and cell death. Central to this disease is the differential processing of the integral transmembrane glycoprotein Amyloid β (Aβ4) precursor protein (APP) that exists as several isoforms (1). The amino acid sequence of APP contains the amyloid domain, which can be released by a two-step proteolytic cleavage (1). β-secretase (BACE) is an aspartic acid proteinase that catalyses the initial step in APP processing by cleaving at the β-secretase cleavage site, generating a membrane-bound, carboxy-terminal β-secretase (BACE) protein. Phospho-GSK-3β (Ser21) (36E9) Rabbit mAb detects endogenous levels of total Tau protein, and also cross-reacts with mouse and rat Tau protein. Since phospho-Tau is a marker for AD, this antibody provides a means of detecting the disease in vivo.

Specificity/Sensitivity: β-Amyloid (D54D2) XP® Rabbit mAb recognizes endogenous levels of total β-Amyloid peptide (Aβ). The antibody detects several isoforms of Aβ, such as Aβ-37, Aβ-38, Aβ-39, Aβ-40, and Aβ-42 regardless of phosphorylation state. APP/β-Amyloid (NAB228) Mouse mAb detects endogenous levels of APP/β-Amyloid protein. Although this antibody recognizes both the phospho and non-phospho forms of the protein, it has been shown to prefer the phosphorylated form in some systems. BACE (D10E5) Rabbit mAb detects endogenous levels of total Neurofilament-L protein. α-Synuclein (Syn204) Mouse mAb detects endogenous levels of total synuclein protein. This antibody detects recombinant α but not β-synuclein (Giasson, B.I. et al., 2000). Tau (TauA6) Mouse mAb detects endogenous levels of total tau protein, and also cross-reacts with MAP2 at 280 kDa. This antibody is predicted to detect all six isoforms of tau based on the amino acid sequence.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human β-amyloid peptide (Aβ), surrounding Glu450 of human Neurofilament-L protein, native bovine tau protein (carboxy terminus), and a synthetic peptide corresponding to residues surrounding Asp490 of human BACE protein, human recombinant α-synuclein protein (amino terminus), β-amyloid protein (amino terminus) (Lee et al., 2003), a synthetic peptide surrounding Gin269 of human γ-secretase protein, or a synthetic phosphopeptide corresponding to residues surrounding Ser21 of human γ-secretase protein.

Background References:
Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% v/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): (#9008) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. 10X Tris Buffered Saline (TBS): (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH₂O, 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 3X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH₂O.
4. 10X Tris-Glycine SDS Running Buffer: (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH₂O, 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 dH₂O, mix.
6. 10X Tris Buffered Saline with Tween® 20 (TBST): (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBS to 900 ml dH₂O, 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.
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 acrate 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 (#7722, 10 µl) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl) 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 cm²) 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.