#8652 Store at -20°C

# AMPA Receptor (GluA) Antibody Sampler Kit

☑ 1 Kit
(6 x 20 µl)

Cell Signaling TECHNOLOGY®

**Orders** ■ 877-616-CELL (2355)
orders@cellsignal.com
**Support** ■ 877-678-TECH (8324)
info@cellsignal.com
**Web** ■ www.cellsignal.com

rev. 09/27/17

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

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|---|---|---|---|
| Phospho-AMPA Receptor (GluA 1) (Ser845) (D10G5) Rabbit mAb | 8084 | 20 µl | 100 kDa | Rabbit IgG |
| Phospho-AMPA Receptor (GluA 2) (Tyr869/Tyr873/Tyr876) Antibody | 3921 | 20 µl | 100 kDa | Rabbit IgG |
| AMPA Receptor (GluA 2) (D39F2) Rabbit mAb | 5306 | 20 µl | 100 kDa | Rabbit IgG |
| AMPA Receptor (GluA 3) (D47E3) Rabbit mAb | 4676 | 20 µl | 100 kDa | Rabbit IgG |
| AMPA Receptor (GluA 4) (D41A11) XP® Rabbit mAb | 8070 | 20 µl | 100 kDa | Rabbit IgG |
| AMPA Receptor (GluA 1) (D4N9V) Rabbit mAb | 13185 | 20 µl | 100 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 AMPA Receptor (GluA) Antibody Sampler Kit provides an economical means of evaluating the four subunits of AMPARs. The kit includes enough primary antibody to perform two western blot experiments with each antibody.

**Background:** AMPA- (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainite-, and NMDA- (N-methyl-D-aspartate) receptors are the three main families of ionotropic glutamate-gated ion channels. AMPA receptors (AMPARs) are comprised of four subunits (GluA 1-4), which assemble as homo- or hetero-tetramers to mediate the majority of fast excitatory transmissions in the CNS. AMPARs are implicated in synapse formation, stabilization, and plasticity (1). AMPARs that lack GluA 2 are permeable to calcium, in contrast to GluR 2-containing AMPARs (2). Post-transcriptional modifications (alternative splicing, nuclear RNA editing) and post-translational modifications (glycosylation, phosphorylation) result in a very large number of permutations, fine-tuning the kinetic properties of AMPARs. Research studies have implicated activity changes in AMPARs in a variety of diseases including Alzheimer's, amyotrophic lateral sclerosis (ALS), stroke, and epilepsy (1).

**Specificity/Sensitivity:** Phospho-AMPA Receptor (GluA 1) (Ser845) (D10G5) Rabbit mAb recognizes endogenous levels of AMPA Receptor (GluA 1) protein only when phosphorylated at Ser845. While the literature refers to this residue as Ser845, it is Ser863 in the UniProt sequence P42261. Phospho-AMPA Receptor (GluA 2) (Tyr869/Tyr873/Tyr876) Antibody detects endogenous levels of GluA 2 protein only when phosphorylated at Tyr869, Tyr873, or Tyr876. It may also detect GluA 3 when phosphorylated at the conserved Tyr880, Tyr884, or Tyr887. These residues are not conserved in GluA 1 or GluA 4. AMPA Receptor (GluA 2) (D39F2) Rabbit mAb detects endogenous levels of total GluA 2 protein. The antibody is not predicted to recognize other AMPA receptor subunits (e.g. GluA 1, GluA 3, or GluA 4) based on sequence homology of the antigen. AMPA Receptor (GluA 3) (D47E3) Rabbit mAb detects endogenous levels of total GluA 3 protein. The antibody is not predicted to detect other AMPA receptor subunits (e.g. GluA 1, GluA 2, or GluA 4) based on sequence homology of the antigen. AMPA Receptor (GluA 4) (D41A11) XP® Rabbit mAb detects endogenous levels of total GluA 4 protein. AMPA Receptor (GluA 1) (D4N9V) Rabbit mAb recognizes endogenous levels of total AMPA Receptor (GluA 1) protein.

**Source/Purification:** Polyclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Tyr869, Tyr873, and Tyr876 of human GluA 2 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

Monoclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human GluA 2 protein, surrounding Pro590 of human GluA 3 protein, surrounding Gln890 of human GluA 4 protein, surrounding Ser845 of human AMPA Receptor (GluA 1) protein or surrounding Ala275 of human AMPA Receptor (GluA 1) protein.

**Background References:**

(1) Palmer, C.L. et al. (2005) *Pharmacol Rev* 57, 253-77.

(2) Cull-Candy, S. et al. (2006) *Curr Opin Neurobiol* 16, 288-97.



**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.

#8652

# 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

**Bovine Serum Albumin (BSA):** (#9998)

**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.

**Biotinylated Protein Ladder Detection Pack:** (#7727)

**Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)

**Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.

**Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)

**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.