PP2A Antibody Sampler Kit

1 Kit (3 x 20 µl)

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

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

Description: The PP2A Antibody Sampler Kit provides an economical means of evaluating PP2A protein. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Protein phosphatase type 2A (PP2A) is an essential protein serine/threonine phosphatase that is conserved in all eukaryotes. PP2A is a key enzyme within various signal transduction pathways as it regulates fundamental cellular activities such as DNA replication, transcription, translation, metabolism, cell cycle progression, cell division, apoptosis and development (1-3). The core enzyme consists of catalytic C and regulatory A (or PR65) subunits, with each subunit represented by α and β isoforms (1). Additional regulatory subunits belong to four different families of unrelated proteins. Both the B (or PR55) and B' regulatory protein families contain α, β, γ, and δ isoforms, with the B' family also including an ε protein. B'' family proteins include PR72, PR130, PR59 and PR48 isoforms, while striatin (PR110) and SG2NA (PR93) are both members of the B'' regulatory protein family. These B subunits competitively bind to a shared binding site on both members of the B''' regulatory protein family. These isoforms, while striatin (PR110) and SG2NA (PR93) are B'' family proteins include PR72, PR130, PR59 and PR48 isoforms, with the B' family also including an α, β, γ, and δ isoforms of the PP2A catalytic subunit and does not cross-react with other PP2A subunits. PP2A C Subunit (52F8) Rabbit mAb detects endogenous levels of the α and β isoforms of the PP2A C subunit and does not cross-react with the B', B'' or B''' families of PP2A subunits. PP2A C Subunit (52F8) Rabbit mAb detects endogenous levels of the α and β isoforms of the PP2A catalytic subunit and does not cross-react with other PP2A subunits.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to the sequence of human PP2A A, B, or C subunit proteins.

Specificity/Sensitivity: PP2A A Subunit (81G5) Rabbit mAb detects endogenous levels of α and β isoforms of the PP2A A subunit and does not cross-react with other PP2A subunits. PP2A B Subunit (100C1) Rabbit mAb detects endogenous levels of the α isoform of PP2A B and may recognize the β, γ, and δ isoforms of PP2A B. The antibody does not cross-react with the B', B'' or B''' families of PP2A B subunits. PP2A B Subunit (100C1) Rabbit mAb detects endogenous levels of the α and β isoforms of the PP2A catalytic subunit and does not cross-react with other PP2A subunits.

Background References:
Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. 1X SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. 10X Tris Buffered Saline (TBS): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. Blocking Buffer: 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. Wash Buffer: 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. Phototope®-HRP Western Blot Detection System #7071: Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

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 per plate of 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 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.