

Affinity-Purified Goat Anti-human/mouse/rat PP1 Inhibitor-2 Antibody

ORDERING INFORMATION

Catalog Number: AF4719

Lot Number: ZCS02

Size: 100 µg (sufficient for 100 mL of blotting solution)

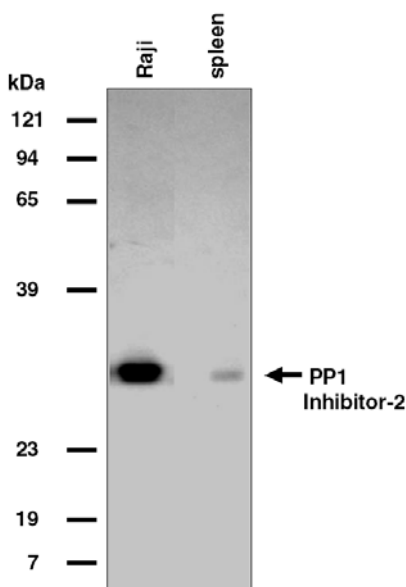
Storage: -20° C

Specificity: human/mouse/rat PP1 Inhibitor-2

Immunogen: *E. coli*-derived recombinant human PP1 Inhibitor-2

Ig Type: goat IgG

Application: Western blot



Detection of PP1 Inhibitor-2 with AF4719.

Lysates from human Raji cells and mouse spleen were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 1 µg/mL goat anti-PP1 Inhibitor-2, as described in *Protocols for Immunoblotting*.

Background

Protein Phosphatase 1 Inhibitor-2 (PP1 Inhibitor-2), also called PP1 regulatory subunit 2 (PP1R2), I2, and IPP2, binds to PP1 through an RVxF consensus sequence. The complex inactivates PP1, preventing it from dephosphorylating substrates such as glycogen phosphorylase. Unlike PP1 Inhibitor-1, the binding of PP1 Inhibitor-2 is Protein Kinase A phosphorylation-independent. Dissociation of the PP1 Inhibitor-2 complex is enhanced by phosphorylation at T72 by GSK3, ERK, or CDK. Overexpression of PP1 Inhibitor-2 in mouse heart improves cardiac function without changing organ size.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived full-length recombinant human PP1 Inhibitor-2 (rhPP1 Inhibitor-2; aa 1 - 205; Accession # P41236). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN₃.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody detects endogenous human, mouse, and rat PP1 Inhibitor-2 at 30 kDa using Western blot. The antibody does not cross-react with recombinant PP1 Inhibitor-1 or DARPP-32.

Application

Western blot - An antibody concentration of 1 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

- Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
- Incubate the membrane overnight at 4° C in Antibody Solution containing 1 µg/mL goat anti-human/mouse/rat PP1 Inhibitor-2.
- Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer.
- Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Catalog # HAF109).
- Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
- Detect with WesternGlo™ Chemiluminescent Detection Reagent (R&D Systems, Catalog # AR004).

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2×10^6 - 1×10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

Optimal dilutions should be determined by each laboratory for each application.