

Affinity-Purified Sheep Anti-human/mouse/rat FKBP13 Antibody

ORDERING INFORMATION

Catalog Number: AF4356

Lot Number: CACX01

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

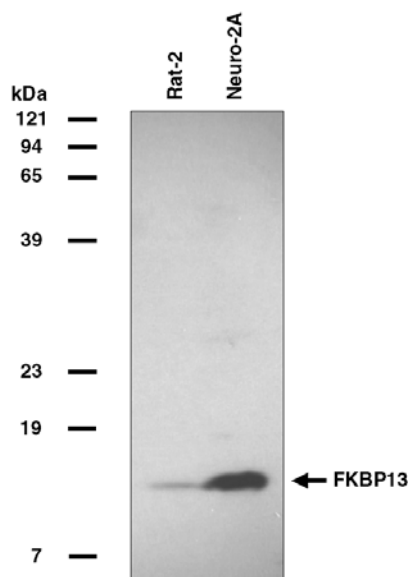
Storage: -20° C

Specificity: human/mouse/rat FKBP13

Immunogen: *E. coli*-derived rhFKBP13 (aa 22 - 142)

Ig Type: sheep IgG

Application: Western blot



Detection of FKBP13 with AF4356.

Lysates from rat Rat-2 and mouse Neuro-2A cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 1 µg/mL sheep anti-FKBP13, as described in *Protocols for Immunoblotting*.

Background

FK506 binding protein, 13 kilodalton molecular weight (FKBP13), also called FKBP2, is a peptidyl-prolyl isomerase that catalyzes the transition between *cis*- and *trans*-proline residues critical for proper folding of proteins. The immunosuppressants FK506 and rapamycin are potent inhibitors of FKBP13. This isomerase is localized in the endoplasmic reticulum and is also associated with cytoskeletal elements such as erythrocyte band 4.1. FKBP13 levels are increased by treatments that affect golgi protein processing such as tunicamycin and heat shock, suggesting that FKBP13 is important in the unfolded protein response observed with endoplasmic reticulum stress.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human FKBP13 (rhFKBP13; aa 22 - 142; Accession # P26885). 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 FKBP13 using Western blot. The antibody does not cross-react with recombinant human FKBP12, FKBP25, FKBP38, FKBP51 or FKBP52.

Application

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

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

Antibody Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 4° C in Antibody Solution containing 1 µg/mL sheep anti-FKBP13.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated donkey anti-sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent Detection Reagent (R&D Systems, Catalog # AR004) or equivalent.

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 2×10^5 - 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.

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1-800-343-7475

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