

ORDERING INFORMATION

Catalog Number: MAB3777

Clone: 422513

Lot Number: CBLL01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Specificity: human/mouse/rat FKBP12

Immunogen: *E. coli*-derived rhFKBP12

Ig class: rat IgG_{2b}

Recommended Application:
Western blot

Background

FK506 binding protein, also called FKBP12 and FKBP1A, was originally characterized as a peptidyl-prolyl isomerase that catalyzes the transition between *cis*- and *trans*-proline residues critical for proper folding of proteins. The macrolide immunosuppressants FK506 (Tacrolimus) and rapamycin bind to FKBP12 with high affinity, while the structurally related compound cyclosporine binds with a much lower affinity. The binding of these drugs causes FKBP12 to become a potent inhibitor of calcineurin phosphatase activity and TOR kinase activity. Knockout mice lacking FKBP12 are morphologically normal, but develop cardiomyopathies that may be related to dysregulation ofryanodine receptors.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived recombinant human FKBP12 (rhFKBP12; aa 2 - 108; Accession # P62942). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

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

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

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 FKBP12 at 12 kDa, and does not cross-react with other FKBP family members using Western blots.

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

2% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

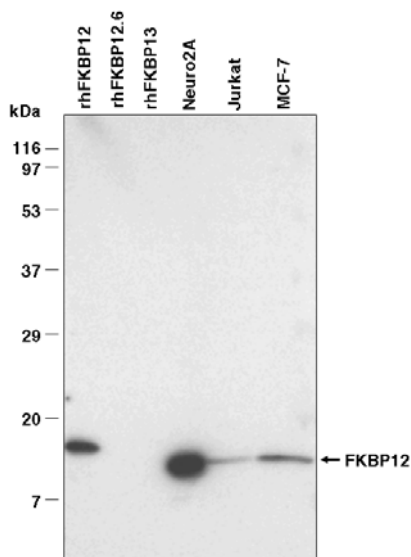
Antibody Solution

2% 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 rat anti-human/mouse/rat FKBP12.
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 goat anti-rat IgG (Zymed).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagent.

Cell lysates for Western blots - 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 x 10⁶ - 1 x 10⁷ 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.



Detection of FKBP12 with MAB3777.

Recombinant human (rh) FKBP12, rhFKBP12.6, rhFKBP13 (5 ng each), and lysates from mouse Neuro2A, human Jurkat and human MCF-7 cells were resolved by SDS-PAGE. Following electrophoresis, proteins were transferred to an Immobilon-P membrane and immunoblotted with 1 µg/mL rat anti-FKBP12, as described in *Protocols for Immunoblotting*.