Monoclonal Anti-human/mouse/rat FKBP25 Antibody

ORDERING INFORMATION

Catalog Number: MAB3955

Clone: 423325

Lot Number: ZVY01

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

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

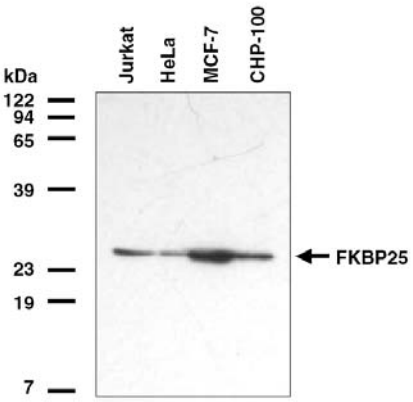
Storage: -20° C

Specificity: human/mouse/rat FKBP25

Immunogen: *E. coli*-derived rhFKBP25

Ig class: rat IgG_{2B}

Recommended Application:
Western blot



Detection of FKBP25 with MAB3955. Lysates from human Jurkat, HeLa, MCF-7, and CHP-100 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 1 μg/mL MAB3955, as described in *Protocols for Immunoblotting.*

Background

FK506 binding protein, 25 kDa molecular weight (FKBP25), also called FKBP3, is a peptidyl-prolyl isomerase that catalyzes the transition between *cis*- and *trans*-proline residues critical for proper folding of proteins. The macrolide immunosuppressant Rapamycin is a potent inhibitor of FKBP25. FKBP25 is localized in the nucleus, where it alters the activity of histone deacetylases and transcription factors. It is found in many tissues, including the brain, thymus, and spleen.

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 full-length recombinant human FKBP25 (rhFKBP25; aa 1 - 224; Accession # Q00688). 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 FKBP25 at 25 kDa. In Western blots it does not show cross-reactivity with recombinant human FKBP12, FKBP12.6, FKBP13, FKBP38, FKBP51, or FKBP52.

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 in	5% nonfat dry milk in
0.15 M NaCl	Blotting Buffer	Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	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 anti-human/mouse/rat FKBP25.
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 1:1000 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 WesternGlo™ Chemiluminescent detection reagents (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 x 10^6 - 1 x 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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