

ORDERING INFORMATION

Catalog Number: MAB4648

Clone: 488411

Lot Number: CBPY01

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 TPT1

Immunogen: *E. coli*-derived rhTPT1

Ig class: mouse IgG₁

Recommended Application:
Western blot

Background

TPT1 (translationally controlled tumor protein 1; also TCTP, HRF and fortilin) is a 23 kDa member of the TCTP family of calcium-binding proteins. It is widely expressed (but not in kidney), and apparently serves as a transcriptional activator, calcium transporter, histamine inducer and antiapoptotic, caspase-3 inhibitor. Human TPT1 is 172 amino acids (aa) in length, with multiple potential splice variants. There is an alternate start site at Met35 that may be accompanied by a deletion of aa 99 - 172. In addition, there may be either a 25 aa or 16 aa C-terminal extension after Cys172. Over aa 1 - 172, human TPT1 is 96% aa identical to mouse TPT1.

Preparation

The antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived full-length recombinant human TPT1 (rhTPT1; aa 1 - 172; Accession # P13693). 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 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

This antibody detects endogenous human, mouse and rat TPT1 at 23 kDa using Western blot.

Application

Western blot - An antibody concentration of 1.0 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

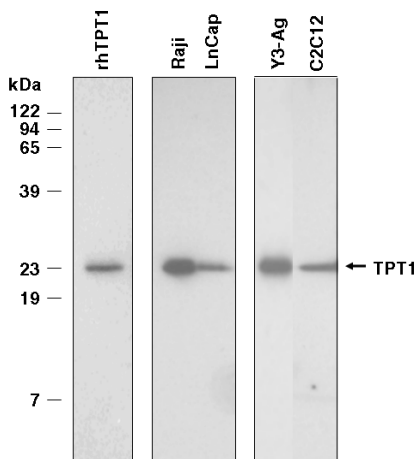
Antibody Solution

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 1.0 µg/mL mouse anti-TPT1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

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.

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



Detection of TPT1 with MAB4648.

Five ng of rhTPT1 and lysates from human Raji, human LnCap, rat Y3-Ag and mouse C2C12 cells were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL anti-TPT1, as described in *Protocols for Immunoblotting*.