

ORDERING INFORMATION

Catalog Number: MAB5815

Clone: 575322

Lot Number: CCYT01

Size: 100 µg

Storage: -20° C

Specificity: human, and mouse
Thioredoxin Reductase 2

Immunogen: *E. coli*-derived rhTRXR2

Ig Class: mouse IgG₁

Recommended Applications:
Western blot
Immunohistochemistry

Background

Thioredoxin Reductase 2 (TRXR2 also known as Thioredoxin Reductase TR3 and Selenoprotein Z) is a 65 kDa, ubiquitously expressed, mitochondrial selenoprotein and member of the class-I pyridine nucleotide-disulfide oxidoreductase family of proteins. Human TRXR2 is synthesized as a 524 amino acid (aa) precursor that contains a 36 aa transit peptide and a 488 aa mature chain. A selenocysteine residue at position 523 is essential for enzymatic activity. Alternate splicing produces four isoforms. Human TRXR2 is 86% and 85% aa identical to mouse and rat TRXR2, respectively. TRXR2 maintains thioredoxin in a reduced state and is implicated in the defense against oxidative stress. It may also play a role in redox-regulated cell signaling.

Preparation

This 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, recombinant human Thioredoxin Reductase 2 (rhTRXR2; Accession # Q9NNW7; aa 268 - 393). 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 100 µL of 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 and mouse TRXR2 at ~63 kDa in Western blots.

Applications

Western blot - An antibody concentration of 0.5 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

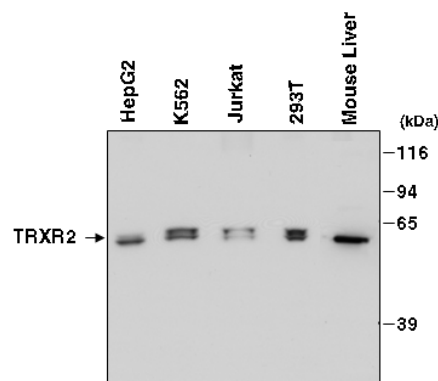
2% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon 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 0.5 µg/mL MAB5815.
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 1:2,000 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 reagent.

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.

Immunohistochemistry - This antibody was used at a concentration of 25 mg/mL with appropriate secondary reagents to detect TRXR2 in paraffin-embedded normal human prostate tissue sections. For chromogenic detection of labeling, the use of R&D Systems Cell and Tissue Staining Kits (CTS Series) is recommended.

For immunohistochemistry images, please refer to our website at
<http://www.RnDSystems.com/go/ihc>.



Detection of TRXR2 with MAB5815.

Lysates from human HepG2, K562, Jurkat and 293T cells and mouse liver tissue were resolved by SDS-PAGE, transferred to Immobilon membranes and immunoblotted with 0.5 µg/mL MAB5815 as described in *Protocols for Immunoblotting*.