**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-Human Thioredoxin

Code No.	Clone	Subclass	Quantity	Concentration
M063-3	2E3	Mouse IgG2a	100 µg	1 mg/mL

BACKGROUND: Thioredoxin (TRX) is a small ubiquitous protein (12 kDa) which is exist in a wide activity of prokaryotic and eukaryotic cells. TRX is activated by reducing its disulfide group (-S-S- → 2SH) between cysteine residues within the conserved active site sequence: -Cys-Gly-Pro-Cys-. The reduction of TRX is specifically catalyzed by "TRX reductase", a FAD and selenocystein containing protein, using NADPH. Once reduced, TRX in turn reduces disulfide bonds of various proteins to regulate the activity of the protein (redox regulation). TRX has multiple biological function; Activation of transcription factors such as NF-κB and glucocotricoid receptor, anti-apoptotic function, stimulation of cytokine expression, tissue protection from ischemia-reperfusion injury, cytoprotection from cytotoxic and DNA-damaging agents, proliferation, and drug resistance in certain malignancies. A part of these functions are due to TRX which exported out of the cell where it has additional ability to stimulate cell growth, cytoprotection, and co-cytokine activity. It is said that some of above TRX functions are related to response against the cellular "stresses". In fact, TRX is translocated from the cytosol into the nucleus and/or highly expressed by various stress such as UV irradiation and viral infection. So, it is suggested that TRX may have a role in the cellular responses against the stresses through the regulation of intracellular redox (oxidation-reduction) atatus.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the recombinant full-length human thioredoxin.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human thioredoxin on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat, HeLa, Raji, U937	NIH/3T3, WR19L, Ba/F3	PC12
Reactivity on WB	+	-	-

INTENDED USE:

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REFERENCES:

1) Xu, W., *et al.*, *PNAS* **103**, 15540-15545 (2006)
2) Andoh, T., *et al.*, *Mol. Pharmacol.* **68**, 1408-1414 (2005)
3) Andoh, T. *et al.*, *J. Biol. Chem.* **278,** 885-890 (2003)
4) Schulze, P. C., *et al.*, *Circ. Res.*, **91**, 689-695 (2002)
5) Liu, Y., *et al.*, *Circ. Res.*, **90**, 1259-1266 (2002)
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7) Tanaka, H., *et al.*, *Vitam. Horm.* **57,** 153-175 (1999)
8) Powis, G., *et al.*, *Chem. Biol. Interact.* **111-112,** 23-34 (1998)
9) Sasada, T., *et al.*, *J. Toxicol. Sci.* **21,** 285-287 (1996)
10) Wollman, E. E., *et al.*, *J. Biol. Chem.* **263,** 15506-15512 (1988)
11) Holmgen, A., *et al.*, *Annu. Rev. Biochem.* **54**, 237-271 (1985)

This antibody is used in reference number 1) - 6).

RELATED PRODUCTS:

K0204-3 Anti-VDUP/Txnip (JY1)
K0205-3 Anti-VDUP/Txnip (JY2)
M013-3 Anti-Thioredoxin (2C9)

PROTOCOL:

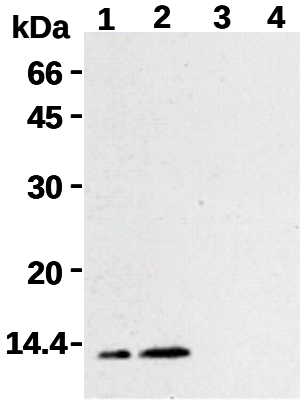
SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up

to 10 seconds).

2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Raji, HeLa)



Western blot analysis of human Thioredoxin expression in Raji (1), HeLa (2), WR19L (3) and PC12 (4) using M063-3.