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

MONOCLONAL ANTIBODY

Anti-Txnip (VDUP1) mAb

Code No.	Clone	Subclass	Quantity	Concentration
K0205-3	JY2	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Thioredoxin (TRX)-binding protein (Txnip), also called Vitamin D3 up-regulated protein 1 (VDUP1) is an endogenous inhibitor of TRX. Redox-dependent regulation of Txnip by mitogenic factors through Reactive oxygen species (ROS) and the specific binding of Txnip to the redox-sensitive cysteine-sulfide center of TRX modulate intracellular levels of ROS and the mitogenic activity of TRX. It has been reported that Txnip plays important roles in diverse cellular processes, including the regulation of cellular redox balance, apoptosis, proliferation, and differentiation.

SOURCE: This antibody was purified from hybridoma (clone JY2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse plasmacytoma cell BIOS/2 with Balb/c mouse splenocyte immunized with human recombinant Txnip (VDUP1).

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 Txnip (VDUP1) (50 kDa) on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 2 µg/200 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested*
*It is reported that clone JY2 can be used in Immunohistochemistry in the reference number 1) and 2).
Immunocytochemistry; Not recommended
Flow cytometry; Not tested

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat*
Cells	Raji, K562, KG1, MRC5, IC2Tr, HEL	P19, WR19L	Not tested
Reactivity on WB	+	+	+*

*Reactivity of this clone to rat Txnip (VDUP1) is described in the reference number 4).

REFERENCES:

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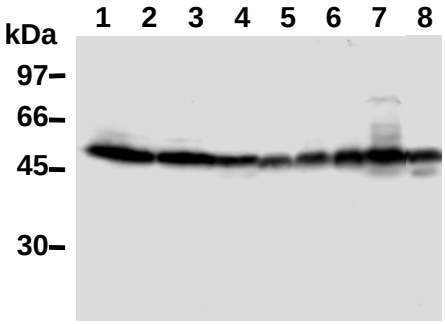
2) Saxena, G., *et al., J. Biol. Chem.* **285**, 3997-4005 (2010) [WB, IHC]
3) Patwari, P., *et al., J. Biol. Chem.* **284**, 24996-25003 (2009) [WB]
4) Chen, J., *et al., Am. J. Physiol. Endocrinol. Metab.* **296**, E1133-E1139 (2009) [WB]
5) Pang, S. T., *et al., J. Mol. Endocrinol.* **42**, 205-214 (2009) [WB]
6) Chen, J., *et al., Diabetes* **57**, 938-944 (2008) [WB]
7) Yoshioka, J., *et al., Circ. Res.*, **101**, 1328-1338 (2007) [WB]
8) Patwari, P., *et al., J. Biol. Chem.* **281**, 21884-21891 (2006)
9) Yoshioka, J., *et al., Circulation* **109**, 2581-2586 (2004)
10) Schulze, P. C., *et al., J. Biol. Chem.* **279**, 30369-30374 (2004)



Western blot analysis of Txnip/VDUP1 expression in Raji (1), K562 (2), KG1 (3), MRC5 (4), IC2Tr (5), HEL (6), P19 (7) and WR19L (8) using K0205-3.

PROTOCOLS:

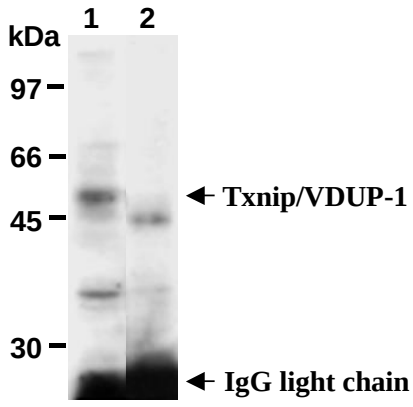
SDS-PAGE & Western Blotting

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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer'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 suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 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, K562, KG1, MRC5, IC2Tr, HEL, P19, WR19L)



Immunoprecipitation of Txnip/VDUP1 from Raji with K0205-3 (1) or mouse IgG1 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with K0205-3.*

Immunoprecipitation

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.
3) Add primary antibody as suggested in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Load 10 μL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
6) 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 manufacturer's manual for precise transfer procedure.
7) 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.
8) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
10) Incubate the membrane with the 1:5,000 HRP-conjugated anti-mouse IgG κ light chain (ZYMED; code no. 04-6620) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
11) Wash the membrane with PBS-T (5 minutes x 6 times).
12) 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.
13) 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 control for Immunoprecipitation; Raji)

RELATED PRODUCTS:

K0205-3	Anti-Txnip (VDUP1) mAb (JY2)
K0204-3	Anti-Txnip (VDUP1) mAb (JY1)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
M063-3	Anti-Thioredoxin (Human) mAb (2E3)
M075-3	Mouse IgG1 (isotype control) (2E12)