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Product datasheet for AM20295AF-N

TXNIP Mouse Monoclonal Antibody [Clone ID: JY1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	JY1
Applications:	IP, WB
Recommended Dilution:	**Western blotting:** 10 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 2 µg/200 µl of cell extract from 5x10e6 cells. Detailed procedure is provided in**Protocols.**
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Human recombinant VDUP-1/Txnip.
Specificity:	This antibody reacts with VDUP-1 (50 kDa) by Western blotting.
Formulation:	PBS, pH 7.2 containing 50% Glycerol without preservatives. State: Azide Free State: Liquid purified IgG fraction.
Concentration:	lot specific
Purification:	Protein-A Agarose Chromatography of hybridoma supernatant.
Conjugation:	Unconjugated
Storage:	Store the antibody (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	thioredoxin interacting protein
Database Link:	Entrez Gene 56338 MouseEntrez Gene 10628 Human Q9H3M7

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Background: Vitamin D3 up-regulated protein 1 (VDUP-1), also called Thioredoxin (TRX)-interacting protein, is an endogenous inhibitor of TRX. Redox-dependent regulation of VDUP-1 by mitogenic factors through Reactive oxygen species (ROS) and the specific binding of VDUP-1 to the redox-sensitive cysteine-sulfide center of TRX modulate intracellular levels of ROS and the mitogenic activity of TRX.

Synonyms: Thioredoxin-interacting protein

Note: This product was originally produced by MBL International.

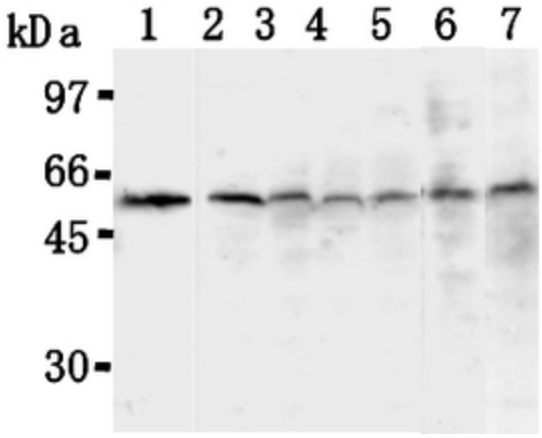
Protocol: **SDS-PAGE & Western Blotting:**
1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate 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 an 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/cm2 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 RT, 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 optimal antibody concentration will depend on the experimental conditions.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1/10,000 HRP-conjugated anti-Mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at RT.
10) Wash the membrane with PBS (5 minutes x 6 times).
11) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes.
13. Develop the film as usual. The conditions for exposure and development may vary.
Positive Controls: Raji, K562, KG1, MRC5, IC2Tr, HEL, P19.

Immunoprecipitation:
1) Wash the cells 3 times with PBS and suspend with 10 volumes 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

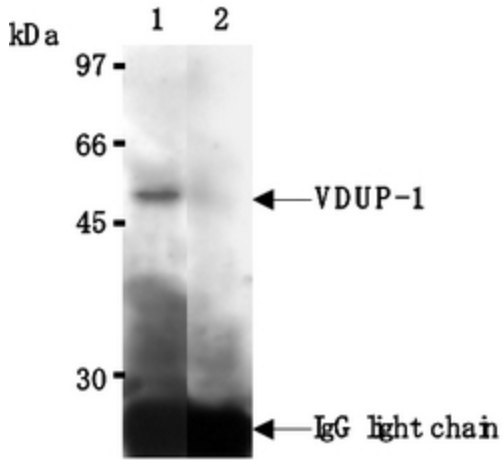
10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A 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 the sample per lane in a 1mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 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 specific 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, pH7.2 containing 1% skimmed milk as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
9) Wash the membrane with PBS (5 minutes x 6 times).
10) Incubate the membrane with the 1:5,000 HRP-conjugated
anti-mouse IgG k light chain diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
11) Wash the membrane with PBS (5 minutes x 6 times).
12) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
13) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.
Positive Control: Raji.

Protein Families: Druggable Genome

Product images:



Western blot analysis of VDUP-1 expression in Raji cells (Lane 1), K562 cells (Lane 2), KG1 cells (Lane 3), MRC5 cells (Lane 4), IC2Tr cells (Lane 5), HEL cells (Lane 6) and P19 cells (Lane 7) using AM20295AF-N VDUP-1 antibody



Immunoprecipitation of VDUP-1 from Raji cells with AM20295AF-N (Lane 1) or Mouse IgG2b (Lane 2). After immunoprecipitation with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM20295AF-N VDUP-1 antibody.