

Product Datasheet

Anti-NEDD4-2/NEDD4L Antibody (orb402251)

Description	Anti-NEDD4-2/NEDD4L Antibody. Tested in ELISA, Flow Cytometry, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Species/Host	Rabbit
Reactivity	Human, Mouse, Rat
Conjugation	Unconjugated
Tested Applications	ELISA, FC, ICC, IHC, WB
Immunogen	E. coli-derived human NEDD4-2 recombinant protein (Position: N703-D975).
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Storage	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
Note	For research use only
Application notes	Western blot, 0.1-0.5µg/ml Immunohistochemistry (Frozen Section), 0.5-1µg/ml Immunocytochemistry, 0.5-1µg/ml Flow Cytometry (Fixed), 1-3µg/1x10 ⁶ cells ELISA, 0.1-0.5µg/ml. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Rabbit IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody
MW	130 kDa
Uniprot ID	Q96PU5

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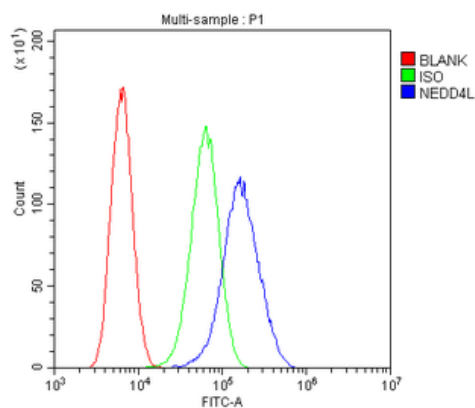
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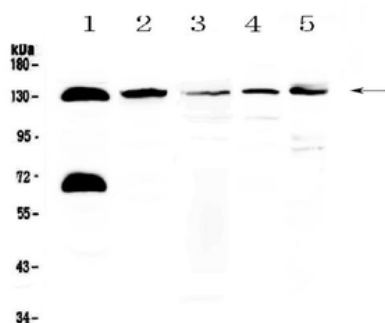
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Expiration Date

12 months from date of receipt.



Flow Cytometry analysis of U2OS cells using anti-NEDD4-2 antibody. Overlay histogram showing U2OS cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NEDD4-2 Antibody (1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



Western blot analysis of NEDD4-2 using anti-NEDD4-2 antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50 μg of sample under reducing conditions. Lane 1: human 22RV1 whole cell lysates, Lane 2: human Jurkat whole cell lysates, Lane 3: rat pancreas tissue lysates, Lane 4: mouse pancreas tissue lysates, Lane 5: mouse NIH3T3 whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-NEDD4-2 antigen affinity purified polyclonal antibody at 0.5 $\mu\text{g}/\text{mL}$ overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for NEDD4-2 at approximately 130KD. The expected band size for NEDD4-2 is at 112KD.

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