

Basic Information

Product Name	Anti-NEDD4 Antibody	
Gene Name	NEDD4	
Source	Rabbit	
Clonality	Polyclonal	
Isotype	IgG	
Species Reactivity	human, rat	
Tested Application	WB, IHC, ICC/IF, FCM, ELISA	
Contents	500 ug/ml antibody with PBS, 0.02% NaN ₃ , 1 mg/ml BSA and 50% glycerol.	
Immunogen	E.coli-derived human NEDD4 recombinant protein (Position: M1-A736).	
Concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	105-150 kDa	
Dilution Ratios	Western blot (WB): 1:500-2000 Immunohistochemistry (IHC): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow Cytometry (Fixed): 1:50-200 Enzyme linked immunosorbent assay (ELISA): 1:100-1000 (Boiling the paraffin sections in 10mM citrate buffer, pH6.0, or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

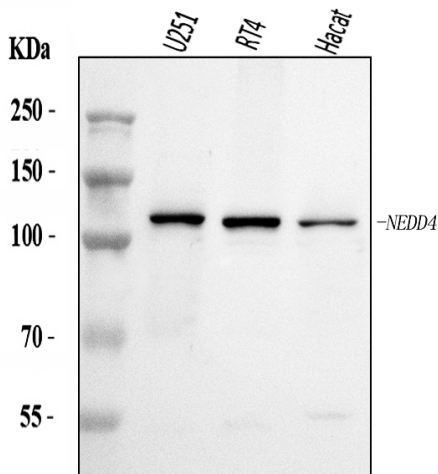
Storage

12 months from date of receipt, -20°C as supplied.

Background Information

E3 ubiquitin-protein ligase NEDD4, also known as neural precursor cell expressed developmentally down-regulated protein 4 (NEDD4), is an enzyme that in humans is encoded by the NEDD4 gene. This gene is the founding member of the NEDD4 family of HECT ubiquitin ligases that function in the ubiquitin proteasome system of protein degradation. The encoded protein contains an N-terminal calcium and phospholipid binding C2 domain followed by multiple tryptophan-rich WW domains and, a C-terminal HECT ubiquitin ligase catalytic domain. It plays critical role in the regulation of a number of membrane receptors, endocytic machinery components and the tumor suppressor PTEN.

Selected Validation Data



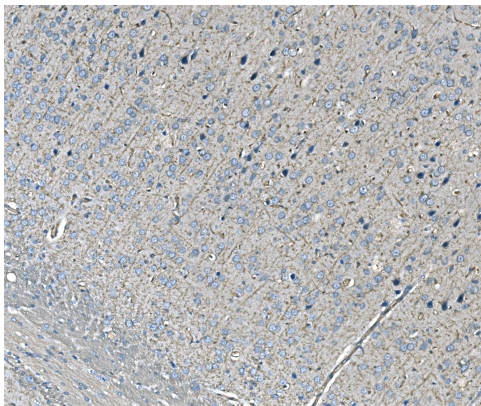
Western blot analysis of NEDD4 using anti-NEDD4 antibody (A00984-3). The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: U251 whole cell lysates,

Lane 2: RT4 whole cell lysates,

Lane 3: Hacat whole cell lysates.

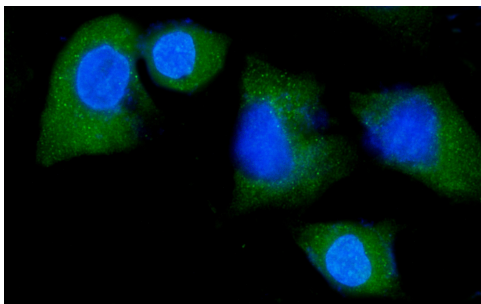
After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-NEDD4 antigen affinity purified polyclonal antibody (A00984-3) at a dilution of 1:1000 and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1197). A specific band was detected for NEDD4 at approximately 105-150 kDa. The expected band size for NEDD4 is at 149 kDa.



IHC analysis of NEDD4 using anti-NEDD4 antibody (A00984-3).

NEDD4 was detected in a paraffin-embedded section of rat brain tissue.

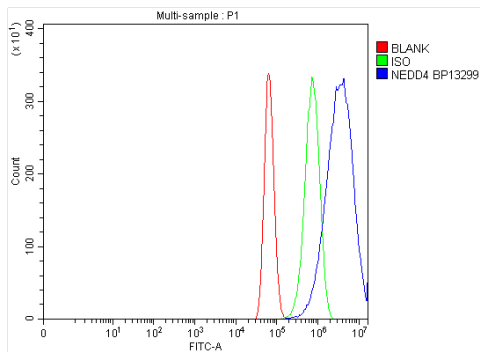
The tissue section was incubated with rabbit anti-NEDD4 Antibody (A00984-3) at a dilution of 1:200 and developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB (Catalog # AR1027) as the chromogen.



IF analysis of NEDD4 using anti-NEDD4 antibody (A00984-3).

NEDD4 was detected in an immunocytochemical section of A549 cells.

The section was incubated with rabbit anti-NEDD4 Antibody (A00984-3) at a dilution of 1:100. DyLight®488 Conjugated Goat Anti-Rabbit IgG (Green) (Catalog # BA1127) was used as secondary antibody. The section was counterstained with DAPI (Catalog # AR1176) (Blue).



Flow Cytometry analysis of U87 cells using anti-NEDD4 antibody (A00984-3).

Overlay histogram showing U87 cells stained with A00984-3 (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 Antibody (A00984-3) at 1:100 dilution for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG at 1:100 dilution used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.