

DESCRIPTION

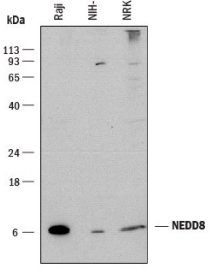
Species Reactivity	Human/Mouse/Rat
Specificity	Detects human NEDD8 in direct ELISAs and Western blots.
Source	Monoclonal Rat IgG _{2B} Clone # 866916
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	<i>E. coli</i> -derived recombinant human NEDD8 Leu2-Gly77 Accession # Q15843
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below

DATA

<p>Western Blot</p> 	<p>Detection of Human, Mouse, and Rat NEDD8 by Western Blot. Western blot shows lysates of Raji human Burkitt's lymphoma cell line, NIH-3T3 mouse embryonic fibroblast cell line, and NRK rat normal kidney cell line. PVDF membrane was probed with 2 µg/mL of Rat Anti-Human/Mouse/Rat NEDD8 Monoclonal Antibody (Catalog # MAB49361) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for NEDD8 at approximately 6 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>NEDD8 in HeLa Human Cell Line. NEDD8 was detected in immersion fixed HeLa human cervical epithelial carcinoma cell line using Mouse Anti-Human/Mouse/Rat NEDD8 Polyclonal Antibody (Catalog # MAB49361) at 25 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

NEDD8 (Neural precursor cell-expressed developmentally down-regulated gene 8; also Rub1) is a 6-8 kDa member of the ubiquitin family of proteins. It is expressed in striated muscle, and via its C-terminal Gly, forms covalent bonds with the cullin family of proteins. This conjugation generates ubiquitin ligase activity that regulates cell cycle proteins. Human proNEDD8 is 81 amino acids (aa) in length and contains one ubiquitin-like domain (aa 1-65), and a 4-5 aa C-terminal propeptide that is cleaved to expose a C-terminal Gly residue that is used to generate a Gly-Lys intermolecular bond. Mature human NEDD8 shows 100% aa identity to NEDD8 from both mouse and rat.