Human/Mouse/Rat NEDD8 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF4936

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat NEDD8 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 2% cross-reactivity with recombinant human NEDD9 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-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.

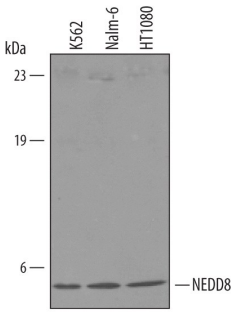
APPLICATIONS

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	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

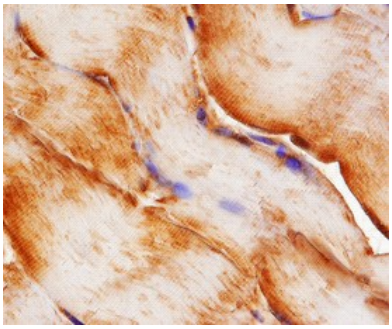
DATA

Western Blot



Detection of Human NEDD8 by Western Blot. Western blot shows lysates of K562 human chronic myelogenous leukemia cell line chronic myelogenous leukemia, Nalm-6 human Pre-B acute lymphocytic leukemia cell line, and HT1080 human fibrosarcoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat NEDD8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4936) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for NEDD8 at approximately 5 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunohistochemistry



NEDD8 in Human Skeletal Muscle. NEDD8 was detected in immersion fixed paraffin-embedded sections of human skeletal muscle using 15 µg/mL Sheep Anti-Human/Mouse/Rat NEDD8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF4936) overnight at 4 °C. Tissue was stained with the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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.** ● 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 cullin family proteins. This conjugation generates ubiquitin ligase activity that regulates cell cycle proteins. Human pro-NEDD8 is 81 amino acids (aa) in length. It 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 sequence identity to NEDD8 from both mouse and canine.

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