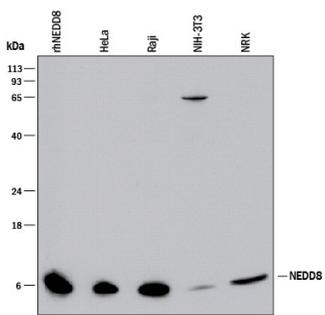
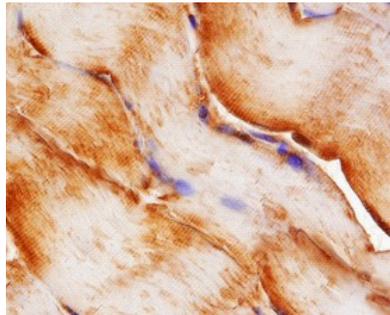


DESCRIPTION	
Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat NEDD8 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 2% cross-reactivity with recombinant human NEDD9 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<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 either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS		
Please Note: Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website.		
	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Immunohistochemistry	5-15 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 Recombinant Human NEDD8, HeLa human cervical epithelial carcinoma cell line, 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 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 6 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p>  <p>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.</p>

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. <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 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.