Recombinant Human APPBP1/UBA3 Complex (NEDD8 Activating Enzyme) His-tag

Catalog Number: E-313B

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human APPBP1/UBA3 Complex protein Ala2-Leu534 with an N-terminal Met and 6-His tag (APPBP1), Met1-Ser463 (UBA3) Accession # Q13564.1(APPBP1) Q8TBC4.2 (UBA3)
Structure / Form	Complex
Predicted Molecular Mass	62 kDa (APPBP1) & 52 kDa (UBA3)

SPECIFICATIONS

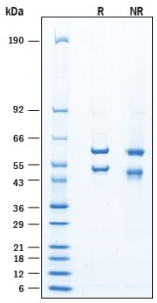
SDS-PAGE	57-63 kDa (APPBP1) & 46-54 kDa (UBA3), under reducing conditions
Activity	Reaction conditions will need to be optimized for each specific application. We recommend an initial Recombinant Human APPBP1/UBA3 Complex (NEDD8 E1) Protein concentration of 50-200 nM.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl, and DTT. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

SDS-PAGE



Recombinant Human APPBP1/UBA3 Complex (NEDD8 Activating Enzyme) His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human APPBP1/UBA3 Complex (NEDD8 Activating Enzyme) His-tag Protein (Catalog # E-313B) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 57-63 kDa (APPBP1) & 46-54 kDa (UBA3), under reducing conditions.

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BACKGROUND

Recombinant Human APPBP1/UBA3 Complex (NEDD8 Activating Enzyme) is a heterodimeric enzyme with a predicted molecular weight of 112 kDa and member of the NEDD8-activating (E1) enzyme family. It is responsible for the first step in the enzymatic cascade that also utilizes a NEDD8-conjugating (E2) enzyme and a NEDD8 ligase (E3) in order to conjugate NEDD8 to protein substrates. The heterodimer is composed of a regulatory subunit, Amyloid beta Precursor Protein Binding Protein 1 (APPBP1), and a catalytic subunit, Ubiquitin-like Modifier Activating Enzyme 3 (UBA3). Human APPBP1 is a 534 amino acid (aa) protein with a predicted molecular weight of 60 kDa that is expressed ubiquitously in fetal tissues and in the adult brain (1). APPBP1 is required for UBA3 neddylation activity, regulates enzyme specificity, and is expressed as two isoforms, the full length protein and a second isoform with an alternate N-terminal, aa1-17, sequence (2). APPBP1 has been shown to drive cell cycle progression, and its expression is increased in the hippocampus of Alzheimer's disease brains (3, 4). Human UBA3 is a 463 aa protein with a predicted molecular weight of 52 kDa. It is ubiquitously expressed and shares high aa sequence identity with the C-terminal domain of human UBE1 (5). UBA3 contains an ATP-binding domain and an active site cysteine residue, Cys237 in humans, which are both common to E1 enzymes. Like APPBP1, two isoforms of UBA3 have been identified in humans, the full length protein and a truncated isoform, which lacks aa 8-21. UBA3 is required for cell cycle progression and has been shown to downregulate steroid receptor activation (4, 6). Neddylation and its associated enzymes have been implicated in the progression of Alzheimer's disease, via neddylation of APP, and cancer via post-translational modification of oncogenes (7, 8).

References:

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