

UBE2F (NCE2) [6His-tagged]

E2 - NEDD8 Conjugating Enzyme

Alternate Names: NEDD8 conjugating enzyme, MGC18120, NCE2

Cat. No. 62-0023-100

Lot. No. 1372

Quantity: 100 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS

Background

The enzymes of the NEDDylation pathway play a pivotal role in a number of cellular processes including the indirect regulation and targeting of substrate proteins for proteosomal degradation. Three classes of enzymes are involved in the process of NEDDylation; the ubiquitin-like activating enzyme APP-BP1/Uba3 (E1), the ubiquitin-like conjugating enzymes (E2s) and protein ligases (E3s). UBE2F is a member of the ubiquitin-like E2 conjugating enzyme family and the human gene was first described by Huang *et al.* (2009). UBE2F acts as a NEDD8 conjugating enzyme both *in vitro* and *in vivo*. UBE2F accepts the ubiquitin-like protein NEDD8 from the Uba3-NAE1 (APP-BP1/Uba3) E1 complex and catalyzes its covalent attachment to other proteins. The specific interaction of UBE2F with the E3 ubiquitin ligase RBX2, but not RBX1, suggests that the RBX2-UBE2F complex NEDDylates specific target proteins such as CUL5, a component of one of the many Cullin Ring Ligases (CRLs) (Huang *et al.*, 2009).

Reference:

Huang DT, Ayrault O, Hunt HW, Taherbhoy AM, Duda DM, Scott DC, Borg LA, Neale G, Murray PJ, Roussel MF, Schulman BA (2009) E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. *Mol Cell* **33**, 483-95.

Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 100 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~25 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

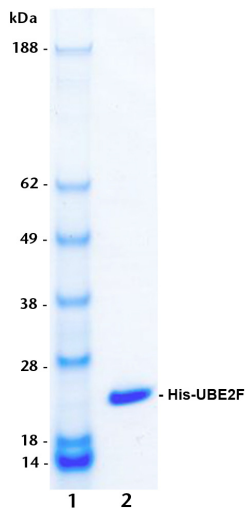
Protein Sequence:

MGSSHHHHHSSGLVPRGSHMASMTG
GQQMGRGS **ML**TLASKLKRDDGLKGSR
TAATASDSTRRVSVRDKLLVKEVALEAN
LPCTCKVHFDPNKLHCFQLTVPDEGYQQ
GKFQFETEVPDAYNMVPPKVKCLTKIWH
NITETGEICLSLLREHSIDGTGWAPTRTLKDV
VWGLNSLFTDLLNFDDPLNIEAAEHHLRDKED
FRNKVDDYIKRYAR

Tag (**bold text**): N-terminal His
Protease cleavage site: Thrombin (LVPR**▼**GS)
UBE2F (regular text): Start **bold italics** (amino acid residues 1-185)
Accession number: NP_542409

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg His-UBE2F



Protein Identification:

Confirmed by mass spectrometry.

E2-NEDD8 Thioester Loading Assay:

The activity of His-UBE2F was validated by loading E1 APP-BP1/Uba3 activated NEDD8 onto the active cysteine of the His-UBE2F E2 enzyme via a transthioesterification reaction. Incubation of the APP-BP1/Uba3 and His-UBE2F enzymes in the presence of NEDD8 and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the NEDD8/His-UBE2F thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0