UBE2F (NCE2) [GST-tagged]

E2 - NEDD8 Conjugating Enzyme

Alternate Names: NEDD8 conjugating enzyme, MGC18120, NCE2

Cat. No.	**62-0024-020**	Quantity:	20 µg
Lot. No.	**30197**	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 proteasomal 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 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: 20 µg

Concentration: 1 mg/ml

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

Molecular Weight: ~48 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

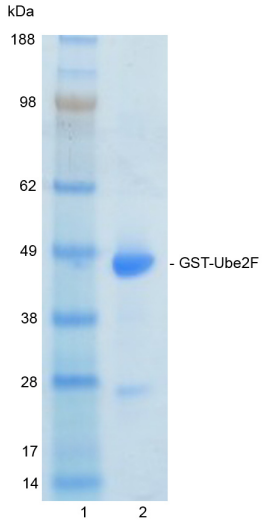
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEM
LKMFEDRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEV
LFQGPLGS***M***LTLASKLKRDDGLKGSRTAATASD
STRRVSVRDKLLVKEVAELEANLPCTCKVHFP
DPNKLHCFQLTVTPDEGYYQGGKFQFETEVP
DAYNMVPPKVKCLTKIWHPNITETGEICLSLL
REHSIDGTGWAPTRTLKDVVWGLNSLFTDLLNFD
DPLNIEAAEHHLRDKEDFRNKVDDYIKRYARD
PLNIEAAEHHLRDKEDFRNKVDDYIKRYAR

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
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 GST-UBE2F



Protein Identification:
Confirmed by mass spectrometry.

E2-NEDD8 Thioester Loading Assay:
The activity of GST-UBE2F was validated by loading E1 APP-BP1/Uba3 activated NEDD8 onto the active cysteine of the GST-UBE2F E2 enzyme via a transthiolation reaction. Incubation of the APP-BP1/Uba3 and GST-UBE2F enzymes in the presence of NEDD8 and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. The sensitivity of this NEDD8/GST-UBE2F thioester bond to the reducing agent DTT was confirmed.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
International: +1-617-245-0003
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +1-617-245-0020
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0