UBE2M (Ubc12) [untagged]

E2 – NEDD8 Conjugating Enzyme

Alternate Names: Nedd8-conjugating enzyme Ubc12, UBC-RS2, UBC12.

Cat. No. **62-0068-100**
Lot. No. **1542**

Quantity: 100 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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). UBE2M is a member of the E2 conjugating enzyme family and the cDNA for human UBE2M was first described by Osaka *et al*. (1998) and shares 42% sequence identity with yeast UBE2M. A trapped ubiquitin like activation complex has been described for the NEDD8 pathway comprising, the E1 APP-BP1/Uba3, two NEDD8 molecules, UBE2M and MgATP. Thioester linkage of NEDD8 to APP-BP1-Uba3 results in an alternate E1 conformation that exposes two NEDD8 binding sites on the E2 enzyme. After transfer of the non-covalently bound NEDD8 to the E2, an alternate E1 conformation allows the release of the thioester bound NEDD8 product. Transference of the NEDD8 thioester linkage between E1 and E2 enzymes in this way can induce a conformational change and alter downstream signalling in the NEDD8 ubiquitin-like (Ubl) cascade (Huang *et al*., 2007).The interaction of different E2 enzymes with different Cullin RING E3 Ligases (CRLs)

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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: ~22 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

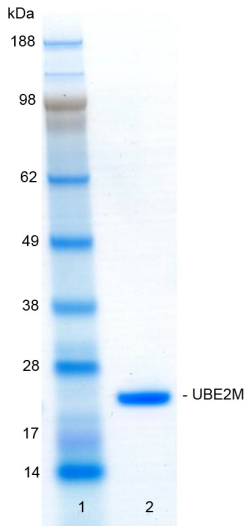
Protein Sequence:

GPGSPEFPGVDSKAAA***M***IKLFSLKQQKKEEE
SAGGTKGSSKKASAAQLRIQKDINELN
LPKTCDISFSDPDDLLNFKLVICPDE
GFYKSGKFVFSFKVGQGYPHDPPKVKCET
MVYHPNIDLEGNVCLNILREDWKPVLTIN
SIIYGLQYLFLEPNPEDPLNKEAAEVLQN
NRRLFEQNVQRSMRGGYIGSTYFERCLK

The residues underlined remain after cleavage and removal of the purification tag.
UBE2M (regular text): Start ***bold italics*** (amino acid residues 1-183)
Accession number: AAH58924

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg UBE2M



Protein Identification:
Confirmed by mass spectrometry.

E2-NEDD8 Thioester Loading Assay:
The activity of UBE2M was validated by loading E1 APP-BP1/Uba3 activated NEDD8 onto the active cysteine of the UBE2M E2 enzyme via a transthiolation reaction. Incubation of the APP-BP1/Uba3 and UBE2M enzymes in the presence of NEDD8 and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the NEDD8/UBE2M thioester NEDD8/UBE2M thioester bond to the reducing agent DTT was confirmed.

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

Lot-specific COA version tracker: v1.0.0

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Background

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has been determined; for example UBE2M/RBX1 and UBE2F/RBX2 can interact with such ligases. This reveals the functional importance of hierarchical expansion of the NEDD8 conjugation system in establishing selective CRL activation (Huang *et al*., 2009). Following ionizing irradiation of a human esophageal cancer cell line a cDNA microarray screen found UBE2M to be upregulated suggesting a role for UBE2M in esophageal cancer (Bo *et al*., 2004).

References:

Bo H, Ghazizadeh M, Shimizu H, Kurihara Y, Egawa S, Moriyama Y, Tajiri T, Kawanami O (2004) Effect of ionizing irradiation on human esophageal cancer cell lines by cDNA microarray gene expression analysis. *J Nippon Med Sch* **71**, 172-80.

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.

Huang DT, Hunt HW, Zhuang M, Ohi MD, Holton JM, Schulman BA (2007) Basis for a ubiquitin-like protein thioester switch toggling E1-E2 affinity. *Nature* **445**, 394-8.

Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, Kawashima S, Tanaka K, Kato S (1998) A new NEDD8-ligating system for cullin-4A. *Genes Dev* **12**, 2263-8.

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