UBE2R1 (UbcH3) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: E2-CDC34, EC 6.3.2.19, Ubiquitin conjugating enzyme E2-32 kDa complementing

Cat. No.	**62-0052-100**	Quantity:	100 µg
Lot. No.	**1380**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteasomal degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). UBE2R1 is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Plon *et al*. (1993). UBE2R1 plays an essential role in promoting the G1-S-phase transition of the eukaryotic cell cycle; it is phosphorylated on serine residues (S203, S222 and S231) present in the acidic tail domain a, region critical for its cell cycle function. Casein Kinase type II (CK2) mediated phosphorylation of UBE2R1 increases ubiquitylation of Sic-1 in the presence of the E3 ligase S-phase kinase-associated protein 1/Cullin/F-box/Cdc4 (SCFCdc4) during cell cycle progression (Sadowski *et al*., 2007). Specific binding of CK2 phosphorylated UBE2R1 to beta-TRCP (β-TRCP) - the substrate recognition unit of the SCF ligase - enhances degradation of its substrate beta-catenin (Semplici *et al*., 2002). UBE2R1 also catalyzes polyubiquitylation of a substrate recruited by the Skp1-Cullin 1-F-box protein-ROC1 E3 ubiquitin ligase. Downregulation of UBE2R1 following let-7 over expression in primary fibro-

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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: ~30 kDa

Purity: >80% by InstantBlue™ SDS-PAGE

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

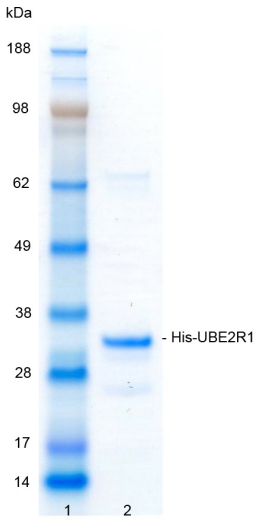
Protein Sequence:

MGSSHHHHHHSSGLVPRGSHMASMTG
GQQMGRGS***A***RPLVPSSQKALLLELK
GLQEEPVEGFRVTLVDEGDLYNWEVAIFGP
PNTYYEGGYFKARLKFPIDYPYSPPAFRFLTK
MWHPNIYETGDVCISILHPPVDDPQSGELPS
ERWNPTQNVRTILLSVISLLNEPNTFSPANV
DASVMYRKWKESKGKDREYTDIIRKQVLGTKV
DAERDGVKVPTTLAEYCVKTKAPAPDEGSDL
FYDDYYEDGEVEEEADSCFGDDEDDSGTEES

Tag (**bold text**): N-terminal His
Protease cleavage site: Thrombin (LVPR▼GS)
UBE2R1 (regular text): Start ***bold italics*** (amino acid residues 2-236)
Accession number: NP_004350

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-UBE2R1



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2R1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2R1 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2R1 enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. The sensitivity of this ubiquitin/His-UBE2R1 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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blasts, results in reduced SCF activity, stabilization of the Wee1 kinase, and an increased fraction of the cells in G(2)/M (Legesse-Miller *et al*., 2009).

References:

Legesse-Miller A, Elemento O, Pfau SJ, Forman JJ, Tavazoie S, Coller HA (2009) let-7 Overexpression leads to an increased fraction of cells in G2/M, direct down-regulation of Cdc34, and stabilization of Wee1 kinase in primary fibroblasts. *J Biol Chem* **284**, 6605-9.

Plon SE, Leppig KA, Do HN, Groudine M (1993) Cloning of the human homolog of the CDC34 cell cycle gene by complementation in yeast. *Proc atl Acad Sci USA* **90**, 10484-8.

Sadowski M, Mawson A, Baker R, Sarcevic B (2007) Cdc34 C-terminal tail phosphorylation regulates Skp1/cullin/F-box (SCF)-mediated ubiquitination and cell cycle progression. *Biochem J* **405**, 569-81.

Semplici F, Meggio F, Pinna LA, Oliviero S (2002) CK2-dependent phosphorylation of the E2 ubiquitin conjugating enzyme UBC3B induces its interaction with beta-TrCP and enhances beta-catenin degradation. *Oncogene* **21**, 3978-87.

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