UBE2R1 (UbcH3) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. **62-0053-100**
Lot. No. **1415**

Quantity: 100 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteosomal 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/Cdc^4 ($SCFCdc^4$) 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 overexpression in primary fibroblasts, 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 Natl 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.

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: ~53 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

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

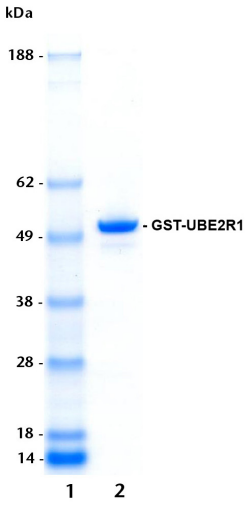
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGS***A***RPLVPSSQKALL
LELKGLQEEPVEGFRVTLVDEGDLYNWEVAIF
GPPNTYYEGGYFKARLKFPIDYPYSPPAFRFLTK
MWHPNIYETGDVCISILHPPVDDPQSGELPS
ERWNPTQNVRTILLSVISLLNEPNTFSPANV
DASVMYRKWKESKGKDREYTDIIRKQVLGTKV
DAERDGVKVPTTLAEYCVKTKAPAPDEGSDLFY
DDYYEDGEVEEEADSCFGDDEDDSGTEES

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
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 GST-UBE2R1



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2R1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2R1 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2R1 enzymes in the presence of ubiquitin and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the ubiquitin/GST-UBE2R1 thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0