UBE2B (HR6B) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: HHR6B, HR6B, RAD6B, Ubiquitin carrier protein B, Ubiquitin protein ligase B

Cat. No. **62-0072-020**
Lot. No. **1823**

Quantity: 20 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 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). UBE2B is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Koken *et al.* (1991). UBE2B shares 70% identity with its yeast homologue but lacks the acidic C-terminal domain. The ring finger proteins RAD5 and RAD18 interact with UBE2B and other members of the RAD6 pathway (Notenboom *et al.*, 2007; Ulrich and Jentsch, 2000). In complex UBE2B and RAD18 trigger replication fork stalling at DNA damage sites during the post replicative repair process (Tsuji *et al.*, 2008). Null mutations of the UBE2B gene in mice are associated with structural abnormalities in sperm and SNP analysis of human UBE2B variants has provided evidence for association of this gene with male infertility (Escalier *et al.*, 2003; Suryavathi *et al.*, 2008).

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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: ~20 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

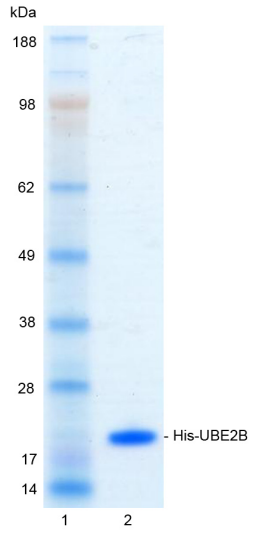
Protein Sequence:

M Y H H H H H H S S G L E V L F Q G P L G S ***S*** T P A R
R R L M R D F K R L Q E D P P V G V S G A P S E N
N I M Q W N A V I F G P E G T P F E D G T
F K L V I E F S E E Y P N K P P T V R F L S K M
F H P N V Y A D G S I C L D I L Q N R W S P T Y D
V S S I L T S I Q S L L D E P N P N S P A N S Q A A Q
L Y Q E N K R E Y E K R V S A I V E Q S W N D S

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2B (regular text): Start ***bold italics*** (amino acid residues 2-152)
Accession number: NP_003328

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-UBE2B



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2B was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2B E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2B 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/His-UBE2B 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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References:

Escalier D, Bai XY, Silvius D, Xu PX, Xu X (2003) Spermatid nuclear and sperm periaxonemal anomalies in the mouse Ube2b null mutant. *Mol Reprod Dev* **65**, 298-308.

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Ulrich HD, Jentsch S (2000) Two RING finger proteins mediate cooperation between ubiquitin-conjugating enzymes in DNA repair. *EMBO J* **19**, 3388-97.

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