# **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).

*Continued on page 2*

## 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

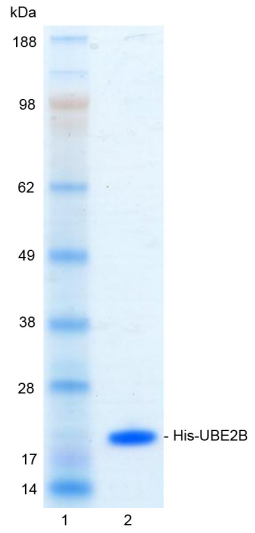
**Protein Sequence:**

**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.

www.ubiquigent.com
Dundee, Scotland, UK

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

**UK HQ and TECHNICAL SUPPORT**
**International:** +44 (0) 1382 381147 (9AM-5PM UTC)
**US/Canada:** +1-617-245-0020 (9AM-5PM UTC)
**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

# **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** | Quantity: | 20 µg |
| **Lot. No.** | **1823** | Storage: | -70˚C |

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS



## Background

*Continued from page 1*

### 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.

Koken MH, Reynolds P, Jaspers-Dekker I, Prakash L, Prakash S, Bootsma D, Hoeijmakers JH (1991) Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. *Proc Natl Acad Sci U S A* **88**, 8865-9.

Notenboom V, Hibbert RG, van Rossum-Fikkert SE, Olsen JV, Mann M, Sixma TK (2007) Functional characterization of Rad18 domains for Rad6, ubiquitin, DNA binding and PCNA modification. *Nucleic Acids Res* **35**, 5819-30.

Suryavathi V, Khattri A, *et al.* (2008) Novel variants in UBE2B gene and idiopathic male infertility. *J Androl* 29, 564-71.

Tsuji Y, Watanabe K, et al. (2008) Recognition of forked and single-stranded DNA structures by human RAD18 complexed with RAD6B protein triggers its recruitment to stalled replication forks. *Genes Cells* **13**, 343-54.

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

www.ubiquigent.com
Dundee, Scotland, UK

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

**UK HQ and TECHNICAL SUPPORT**
**International:** +44 (0) 1382 381147 (9AM-5PM UTC)
**US/Canada:** +1-617-245-0020 (9AM-5PM UTC)
**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