UBE2A (HR6A) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: HHR6A, HR6A, RAD6A, UBC2, EC 6.3.2.19, Ubiquitin-conjugating enzyme E2A

Cat. No.	**62-0071-100**	Quantity:	100 µg
Lot. No.	**1822**	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 the 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). UBE2A is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Koken *et al.* (1991). UBE2A shares 70% identity with its yeast homologue but lacks the acidic C-terminal domain. The ring finger proteins RAD5 and RAD18 interact with UBE2A and other members of the RAD6 pathway (Ulrich and Jentsch, 2000). Phosphorylation of UBE2A by CDK1 and 2 increases its activity during the G2/M phase of the cell cycle (Sarcevic *et al.*, 2002). UBE2A is required for post-replicative DNA damage repair in eukaryotic cells and it is thought binding to ZNF198 may be involved in this process (Kunapuli *et al.*, 2003). A nonsense mutation resulting in the loss of a 25 amino acid region in the C-terminal domain of UBE2A has been identified as a cause of a novel X-linked mental retardation (XLMR) syndrome (Nascimento *et al.*, 2006).

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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: ~21 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

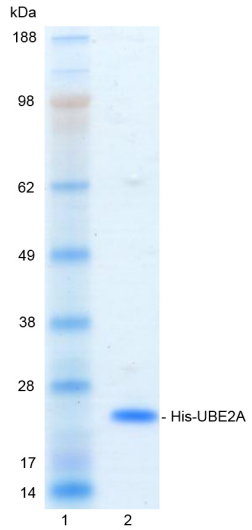
Protein Sequence:

MGSSHHHHHHSSGLVPRGSHMASMTG
GQQMGRDPNSSSVD***S***TPARRRLMRD
FKRLQEDPPAGVSGAPSENNIMVW
NAVIFGPEGTPFEDGTFKLTIEFT
EEYPNKPPTVRFVSKMFHPNVY
ADGSICLDILQNRWSPTYDVSSILT
SIQSLLDEPNPNSPANSQAAQLYQENK
REYEKRVSAIVEQSWRDC

Tag (**bold text**): N-terminal His
Protease cleavage site: Thrombin (LVPR▼GS)
UBE2A (regular text): Start ***bold italics*** (amino acid residues 2-152)
Accession number: NP_003327

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-UBE2A



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2A was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2A E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2A 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-UBE2A 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

Continued from page 1

References:

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.

Kunapuli P, Somerville R, Still IH, Cowell JK (2003) ZNF198 protein, involved in rearrangement in myeloproliferative disease, forms complexes with the DNA repair-associated HHR6A/6B and RAD18 proteins. *Oncogene* **22**, 3417-23.

Nascimento RM, Otto PA, de Brouwer AP, Vianna-Morgante AM (2006) UBE2A, which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome. Am *J Hum Genet* **79**, 549-55.

Sarcevic B, Mawson A, Baker RT, Sutherland RL (2002) Regulation of the ubiquitin-conjugating enzyme hHR6A by CDK-mediated phosphorylation. *EMBO J* **21**, 2009-18.

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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