UBE2A (HR6A) [untagged]

E2 – Ubiquitin Conjugating Enzyme

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

The enzymes of the ubiquitylation

pathway play a pivotal role in a num-

ber of cellular processes including

the regulated and targeted protea-

somal degradation of substrate pro-

teins. Three classes of enzymes are

involved in the process of ubiquitylation; activating enzymes (E1s), con-

jugating 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 veast 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 dur-

ing the G2/M phase of the cell cycle

(Sarcevic et al., 2002). UBE2A is re-

guired for post-replicative DNA dam-

age repair in eukaryotic cells and it is

thought binding to ZNF198 may be in-

volved 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).

Cat. No.	62-0002-020
Lot. No.	30118

Quantity: 20 µg Storage: -70°C

NOT FOR USE IN HUMANS

FOR RESEARCH USE ONLY

Background

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: ~17 kDa

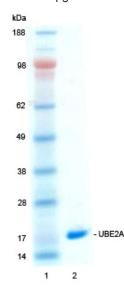
Purity: >95% by InstantBlue™ SDS-PAGE

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

Quality Assurance

Purity:

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





CERTIFICATE OF ANALYSIS Page 1 of 2

Protein Sequence:

<u>GPLGSPNSRVD</u>STPARRRLMRDFKRLQEDP PAGVSGAPSENNIMVWNAVIFGPEGTPFEDGT FKLTIEFTEEYPNKPPTVRFVSKMFHPNVY ADGSICLDILQNRWSPTYDVSSILTSIQSLL DEPNPNSPANSQAAQLYQENKREYEKRV SAIVEQSWRDC

The residues <u>underlined</u> remain after cleavage and removal of the purification tag. UBE2A (regular text): Start **bold italics** (amino acid residues 2-152) Accession number: NP_003327

Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of UBE2A was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the UBE2A E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and 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/UBE2A thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0

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Quantity:

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

References:

Cat. No.

Lot. No.

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 USA* **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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