UBE2A (HR6A) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. **62-0001-020**
Lot. No. **1384**

Quantity: 20 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 the 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). 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).

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 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.

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: ~45 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

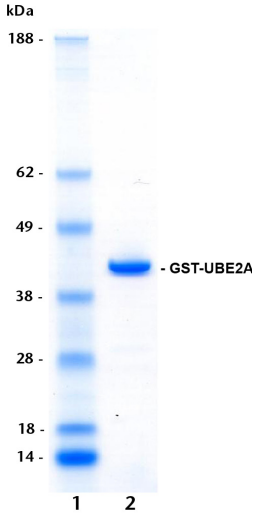
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYYIDGD VKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP DFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG GGDHPPKSDLEVLFQGPLGSPNSRVD***S***TPAR RRLMRDFKRLQEDPPAGVSGAPSENNIMVW NAVIFGPEGTPFEDGTFKLTIEFTEEYPNKPPT VRFVSKMFHPNVYADGSICLDILQNRWSPTYD VSSILTSIQSLLDEPNPNSPANSQAAQLYQENK REYEKRVSAIVEQSWRDC

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
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 GST-UBE2A



Protein Identification:
Confirmed by mass spectrometry.

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