UBE2I (Ubc9) [6His-tagged]

E2 - SUMO Conjugating Enzyme

Alternate Names: P18, SUMO-1 protein ligase, UBC9, Ubiquitin conjugating enzyme UbcE2A, Ubiquitin like protein SUMO-1 conjugating enzyme

Cat. No. **62-0033-020**
Lot. No. **1374**

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 SUMOylation pathway play a pivotal role in a number of cellular processes including nuclear transport, signal transduction, stress responses and cell cycle progression. Covalent modification of proteins by small ubiquitin-related modifiers (SUMOs) may also modulate their stability and subcellular compartmentalisation. Three classes of enzymes are involved in the process of SUMOylation; an activating enzyme (E1), conjugating enzyme (E2) and protein ligases (E3s). UBE2I is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Wang *et al.* (1996). The human UBE2I cDNA contains 7 exons sharing 56% and 100% identity with the yeast and mouse homologues respectively (Nacerddine *et al.*, 2005; Shi *et al.*, 2000; Wang *et al.*, 1996). The candidate tumor suppressor gene Fragile Histidine Triad (FHIT) located on 3p14.2 is deleted in many types of human cancer. UBE2I binds to FHIT and this interaction is thought to be involved in the degradation of S and M phase cyclins and cell cycle control. Proliferating Cell Nuclear Antigen (PCNA) a DNA polymerase sliding clamp involved in DNA synthesis and repair is a substrate for UBE2I. SUMOylation of PCNA is mediated by UBE2I and occurs on a specific lysine residue - K146 - which may also be modified by ubiquitin (Hoege *et al.*, 2002). Crystallography has revealed that UBE2I forms part of a 4 protein complex consisting of a NUP358/RANBP2 E3 ligase domain, and SUMO1 conjugated to the carboxy-terminal domain of RANGAP1. A model for the complex has been proposed in which NUP358/RANBP2 acts as an E3 by binding both SUMO and UBE2I to position the SUMO-E2-thioester in an optimal orientation to enhance conjugation (Reverter and Lima, 2005). SUMOylation of Amyloid Precursor Protein (APP) is

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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

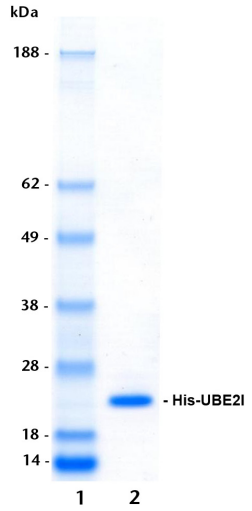
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MGSSHHHHHHSSGLEVLFQGPGS***M***SGIALSR
LAQERKAWRKDHPFGFVAVPTKNPDGTMN
LMNWECAIPGKKGTPWEGGLFKLRMLFKD
DYPSSPPKCKFEPPLFHPNVYPSGTVCLSILEED
KDWRPAITIKQILLGIQELLNEPNIQDPAQAEAY
TIYCQNRVEYEKRVRAQAKKFAPS

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2I (regular text): Start ***bold italics*** (amino acid residues 1-158)
Accession number: NP_003336

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg His-UBE2I



Protein Identification:
Confirmed by mass spectrometry.

SUMO-E2 Thioester Loading Assay:
The activity of His-UBE2I was validated by loading E1 SAE1/SAE2 activated SUMO onto the active cysteine of the His-UBE2I E2 enzyme via a transthiolation reaction. Incubation of the SAE1/SAE2 and His-UBE2I enzymes in the presence of SUMO and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the SUMO/His-UBE2I 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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reported to be associated with decreased levels of beta amyloid (Abeta) aggregates, suggesting a role in the pathogenesis of Alzheimer's Disease (AD). An investigation into single nucleotide polymorphisms (SNPs) in the UBE2I gene have shown an association between this and the risk of late onset AD (Ahn *et al.*, 2009).

References:

Ahn K, Song JH, Kim DK, Park MH, Jo SA, Koh YH (2009) Ubc9 gene polymorphisms and late-onset Alzheimer's disease in the Korean population: a genetic association study. *Neurosci Lett* **465**, 272-5.

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* **419**, 135-41.

Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A (2005) The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. *Dev Cell* **9**, 769-79.

Reverter D, Lima CD (2005) Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. *Nature* **435**, 687-92.

Shi Y, Zou M, Farid NR, Paterson MC (2000) Association of FHIT (fragile histidine triad), a candidate tumour suppressor gene, with the ubiquitin-conjugating enzyme hUBC9. *Biochem J* **352** Pt 2, 443-8.

Wang ZY, Qiu QQ, Seufert W, Taguchi T, Testa JR, Whitmore SA, Callen DF, Welsh D, Shenk T, Deuel TF (1996) Molecular cloning of the cDNA and chromosome localization of the gene for human ubiquitin-conjugating enzyme 9. *J Biol Chem* **271**, 24811-6.

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