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Catalog #	Aliquot Size
U224-380H-20	20 µg
U224-380H-50	50 µg

UBE2I (UBC9), Active

Recombinant full-length human proteins expressed in *E. coli* cells

Catalog # U224-380H

Lot # D2592-6

Product Description

Recombinant full length human UBE2I was expressed in E. coli cells using an N-terminal His tag. The gene accession number is NM_003345.

Gene Aliases

C358B7.1; P18; UBC9

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

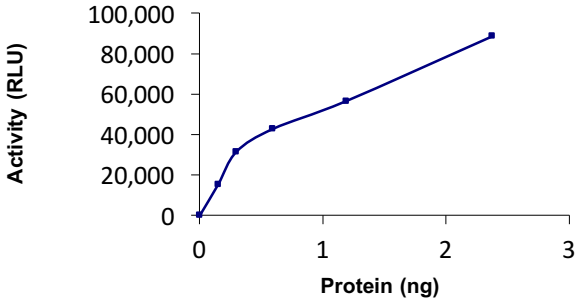
Scientific Background

UBE2I or ubiquitin-conjugating enzyme E2I is a member of the E2 ubiquitin-conjugating enzyme family which is required for post-replicative DNA damage repair. UBE21 also known as UBC9, plays a role DNA damage repair via interaction with WT1, which is able to impose a block in cell cycle progression in eukaryotic cells (1). Listeria and probably other pathogens, dampens the host response by decreasing the sumoylation level of proteins critical for infection by targeting UBC21, an essential enzyme of the SUMO pathway. UBC21 and the SUMO pathway are crucial for proper nuclear architecture, accurate chromosome segregation, and embryonic viability (2).

References

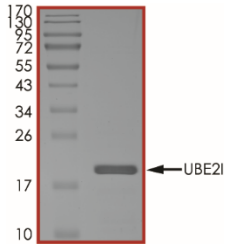
1. Wang, Z.-Y. et.al: Molecular cloning of the cDNA and chromosome localization of the gene for human ubiquitin-conjugating enzyme 9. J. Biol. Chem. 271: 24811-24816, 1996.
2. Nacerddine, K. et.al The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev. Cell 9: 769-779, 2005.

Specific Activity



The specific activity of UBE2I was determined to be **200 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UBE2I was determined to be **>95%** by densitometry, approx. MW **19 kDa**.

UBE2I (UBC9), Active

Recombinant full-length human protein expressed in *E. coli* cells

Catalog #	U224-380H
Specific Activity	200 nmol/min/mg
Lot #	D2592-6
Purity	>95%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Enzymes

Active UBE2I (Catalog #: U224-380H) and SAE1/UBA2 (Catalog #: U208-380G) diluted with SUMOylation Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active UBE2I for optimal results).

SUMOylation Buffer

Buffer components: 50mM Tris-HCl (pH7.5), 5mM $MgCl_2$. Add 0.5mM DTT prior to use.

AMP-Glo™ Assay (Promega, Catalog #: V5011)

AMP, 10 mM
Ultra Pure ATP, 10mM
AMP-Glo™ Reagent I
AMP-Glo™ Reagent II
Kinase-Glo™ One Solution

Substrate

Human SUMO2 (1-93) (Catalog #: S294-31H) and RanGAP1 (Catalog #: R298-31H) diluted with SUMOylation Buffer to appropriate working stocks.

Assay Protocol

The UBE2I assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin-like (UBL) protein conjugation is proportional to the amount of generated AMP, and the presence of all components of the UBLs conjugation machinery (UBL, E1, E2 and substrate) is required for maximal activity of the system.

Step 1. Thaw the active UBE2I, SAE1/UBA2 and SUMO2, RanGAP1 on ice, and all AMP-Glo™ components except AMP-Glo™ Reagent II at room temperature. Keep AMP-Glo™ Reagent II on ice.

Step 2. Prepare the following working solutions with Ubiquitination Buffer:

- 2X Reaction Cocktail: 460ng/μl SUMO2 + 7ng/μl SAE1/UBA2 + 80ng/μl RanGAP1 + 50μM ATP
- 2X final concentration of Active UBE2I

Step 3. In a half-area white 96-well plate, add the following components to bring the initial reaction volume to 10 μl:

Component 1. 5 μl of 2X Reaction Cocktail

Component 2. 5 μl of 2X Active UBE2I

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of Ubiquitination Buffer.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Seal the plate with a plate seal and incubate at 30°C for 2 hours

Step 5. Equilibrate plate to room temperature. Add 10 μl of AMP-Glo™ Reagent I to all wells, mix by shaking for 1-2 minutes. Incubate the plate at room temperature for 60 minutes.

Step 6. Prepare AMP Detection Solution by adding AMP-Glo™ Reagent II to Kinase-Glo™ One Solution at a 1:100 volume ratio. Add 20 μl of the Detection Solution to all wells. Mix for 1-2 minutes and incubate at room temperature for 30 minutes

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 8. Using the AMP standard curve, determine the concentration of AMP produced (μM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine AMP amount from RLUs, see AMP-Glo™ Assay protocol at Promega's website: www.promega.com/protocols

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AMP](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$