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Catalog #	Aliquot Size
U217-380H-20	20 µg
U217-380H-50	50 µg

UBE2E1 (UBCH6), Active

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

Catalog # U217-380H
Lot # D2473-7

Product Description

Recombinant human UBE2E1 (2-end) was expressed in E. coli cells using an N-terminal His tag. The gene accession number is NM_003341.

Gene Aliases

UBCH6

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.

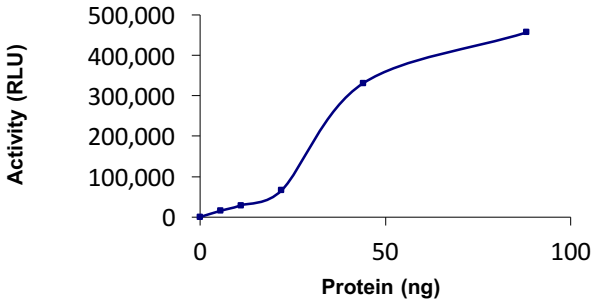
Scientific Background

Ubiquitin-conjugating enzyme E2 E1 (UBE2E1) belongs to the ubiquitin-conjugating enzyme family. It accepts ubiquitin activated by the E1 complex and catalyzes its covalent attachment to short-lived and abnormal proteins, and mediates their selective degradation. It was also found to be responsible for 'Lys-48'-linked polyubiquitination in vitro.

References

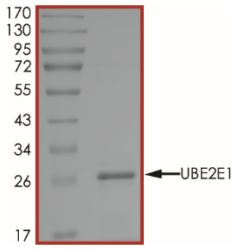
1. David Y, et al. The E2 ubiquitin-conjugating enzymes direct polyubiquitination to preferred lysines. J Biol Chem. 2010 19; 285:8595-604.
2. http://www.uniprot.org/uniprot/P51965

Specific Activity



The specific activity of UBE2E1 was determined to be **3 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>95%** by densitometry. approx. MW **27kDa**.

UBE2E1 (UBCH6), Active

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

Catalog #	U217-380H
Specific Activity	3 nmol/min/mg
Lot #	D2473-7
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 Ubiquitinating Enzymes

Active UBE2E1 (Catalog #: U217-380H), UBA1 (Catalog #: U201-380G) and BIRC7 (Catalog #: B281-380G) diluted with Ubiquitination 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 UBE2E1 for optimal results).

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

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM $MgCl_2$, 0.1mg/ml BSA. Add 0.5mM DTT prior to use.

Substrate (Catalog #: U06-54N)

Wild-type ubiquitin protein diluted with Ubiquitination Buffer to appropriate working stock.

Assay Protocol

The UBE2E1 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 UBE2E1, UBA1, BIRC7 and ubiquitin 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: 170ng/μl ubiquitin + 15ng/μl UBA1 + 48ng/μl BIRC7 + 50μM ATP
- 2X final concentration of Active UBE2E1

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 UBE2E1

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)

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

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