

UBE2E3 (UBCH9), Active

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

Catalog # U219-380H

Lot # V2408-12

Product Description

Recombinant full-length human UBE2E3 was expressed in *E. coli* cells using an N-terminal His tag. The UBE2E3 gene accession number is [NM_006357](#).

Gene Aliases

UBCH9; UbcM2

Formulation

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

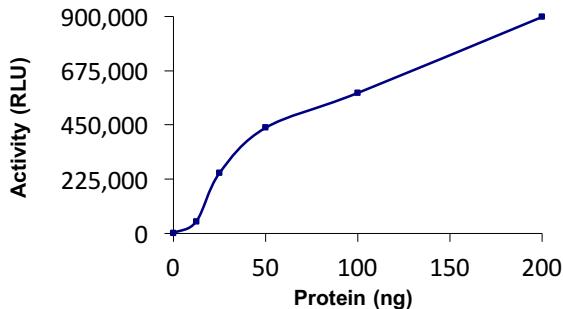
Scientific Background

UBE2E3 also known as UBCH9 or ubiquitin-conjugating enzyme E2E 3 is a member of the E2 ubiquitin-conjugating enzyme family. UBE2E3 is highly expressed in skeletal muscle. UBE2E3 is essential for the proliferation of RPE-1 cells and is downregulated during RPE layer maturation in the developing mouse eye. These findings indicate that UBE2E3 is a major enzyme in modulating the balance between RPE cell proliferation and differentiation.

References

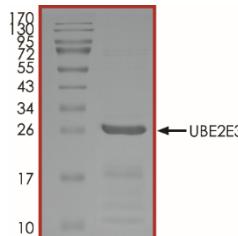
1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. *Anal. Biochem.* 510: 41-51, 2016
2. Matuschewski, K. et al: Identification of a novel family of ubiquitin-conjugating enzymes with distinct amino-terminal extensions. *J. Biol. Chem.* 271: 2789-2794, 1996
3. Ito, K. et al: cDNA cloning, characterization, and chromosome mapping of UBE2E3 (alias UbcH9), encoding an N-terminally extended human ubiquitin-conjugating enzyme. *Cytogenet. Cell Genet.* 84: 99-104, 1999

Specific Activity



The specific activity of UBE2E3 was determined to be **17 nmol/min/mg** as per activity assay protocol.

Purity



The purity of UBE2E3 was determined to be >95% by densitometry, approx. MW **25 kDa**.

UBE2E3 (UBCH9), Active

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

Catalog #	U219-380H
Specific Activity	17 nmol/min/mg
Lot #	V2408-12
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 UBE2E3 (Catalog #: U219-380H), UBA1 (Catalog #: U201-380G) and BIRC3 (Catalog #: B280-380G) 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 UBE2E3 for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM MgCl₂, 0.1mg/ml BSA. 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 (Catalog #: U06-54N)

Wild-type ubiquitin protein diluted with Ubiquitination Buffer to a working stock of 170ng/μl (20μM).

Assay Protocol

The UBE2E3 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin conjugation is proportional to the generated AMP, and the presence of all components of the Ub conjugation machinery (Ub, E1, E2, and E3) is required for maximal activity of the system.

- Step 1.** Thaw the active UBE2E3, UBA1, BIRC3 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:
 - o 2X Reaction Cocktail: 170ng/μl ubiquitin + 15ng/μl UBA1 + 40ng/μl BIRC3 + 50μM ATP
 - o 2X final concentration of Active UBE2E3
- 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 UBE2E3

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 37°C for 60 minutes
- 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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