

UBE2G1 (UBC7), Active

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

Catalog # U221-380H

Lot # V2408-13

Product Description

Recombinant full-length human UBE2G1 was expressed in *E. coli* cells using an N-terminal His tag. The UBE2G1 gene accession number is [NM_003342](#).

Gene Aliases

UBC7; E217K; UBE2G

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.

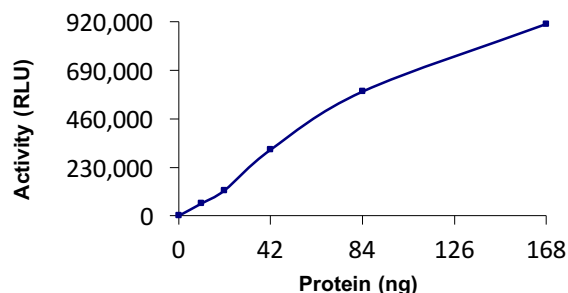
Scientific Background

UBE2G1 (UBC7) or ubiquitin-conjugating enzyme E2G 1 is a member of the E2 ubiquitin-conjugating enzyme family and catalyzes the covalent attachment of ubiquitin to other proteins which are involved in degradation of muscle-specific proteins. UBC7 is highly expressed in skeletal muscle. UBC7 is involved in the selective ubiquitylation of different substrates by the same E3 complex.

References

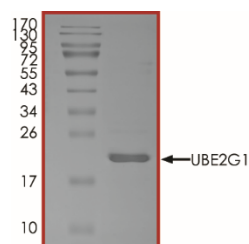
1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. *Anal. Biochem.* 510: 41-51, 2016
2. Watanabe, T.K. et al: Molecular cloning of UBE2G, encoding a human skeletal muscle-specific ubiquitin-conjugating enzyme homologous to UBC7 of *C. elegans*. *Cytogenet. Cell Genet.* 74: 146-148, 1996
3. David, Y. et al: The E2 ubiquitin-conjugating enzymes direct polyubiquitination to preferred lysines. *Cytogenet. J Biol Chem.* 285(12): 8595-8604, 2010

Specific Activity



The specific activity of UBE2G1 was determined to be **11 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UBE2G1 was determined to be **>95%** by densitometry, approx. MW **21 kDa**.

UBE2G1 (UBC7), Active

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

Catalog #	U221-380H
Specific Activity	11 nmol/min/mg
Lot #	V2408-13
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.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes

Active UBE2G1 (Catalog #:U221-380H), 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 UBE2G1 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 UBE2G1 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 UBE2G1, 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 UBE2G1
- 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 UBE2G1

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)

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

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
www.signalchem.com

FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.