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Catalog #	Aliquot Size
U232-380G-20	20 µg
U232-380G-50	50 µg

UBE2O, Active

Recombinant full-length human proteins expressed in *Sf9* cells

Catalog # U232-380G

Lot # V2408-16

Product Description

Recombinant full-length human UBE2O was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The UBE2O gene accession number is BC022237.

Gene Aliases

E2-230K, FLJ12878, KIAA1734

Formulation

Recombinant proteins stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 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.

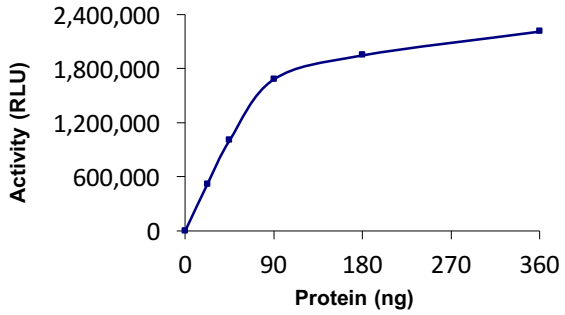
Scientific Background

UBE2O or ubiquitin-conjugating enzyme E2O is a part of the ubiquitin-mediated protein degradation pathway in which a thiol-ester linkage forms between a conserved cysteine and the C-terminus of ubiquitin and complexes with ubiquitin. HIV-1 Tat has been identified to have a physical interaction with ubiquitin-conjugating enzyme E2O (UBE2O) in human HEK293 and Jurkat cell lines by using affinity tagging and purification mass spectrometry analyses.

References

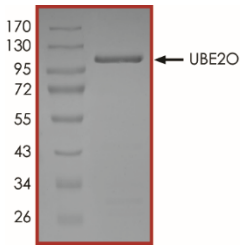
1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. Anal. Biochem. 510: 41-51, 2016
2. Zhang, X. et al: Fine-tuning BMP7 signalling in adipogenesis by UBE2O/E2-230K-mediated monoubiquitination of SMAD6. EMBO J. 32(7):996-1007, 2013
3. Mashtalir, N. et al: Autodeubiquitination protects the tumor suppressor BAP1 from cytoplasmic sequestration mediated by the atypical ubiquitin ligase UBE2O. Mol. Cell. 54(3):392-406, 2014

Specific Activity



The specific activity of UBE2O was determined to be **25 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UBE2O was determined to be **>95%** by densitometry, approx. MW **106 kDa**.

UBE2O, Active

Recombinant full-length human protein expressed in *Sf9* cells

Catalog #	U232-380G
Specific Activity	25 nmol/min/mg
Lot #	V2408-16
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
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FOR IN VITRO RESEARCH PURPOSES ONLY. NOT INTENDED FOR USE IN HUMAN OR ANIMALS.

Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes

Active UBE2O (Catalog #: U232-380G), 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 UBE2O for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM $MgCl_2$, 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 UBE2O 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 UBE2O, 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:

- 2X Reaction Cocktail: 170ng/µl ubiquitin + 15ng/µl UBA1 + 40ng/µl BIRC3 + 50µM ATP
- 2X final concentration of Active UBE2O

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 UBE2O

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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