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Catalog #	Aliquot Size
U210-380H-20	20 µg
U210-380H-50	50 µg

UBE2A, Active

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

Catalog # U210-380H
Lot # V2408-7

Product Description

Recombinant full-length human UBE2A was expressed in *E. coli* cells using an N-terminal His tag. The UBE2A gene accession number is NM_003336.

Gene Aliases

HHR6A; MRXS30; MRXSN; RAD6A; UBC2

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.

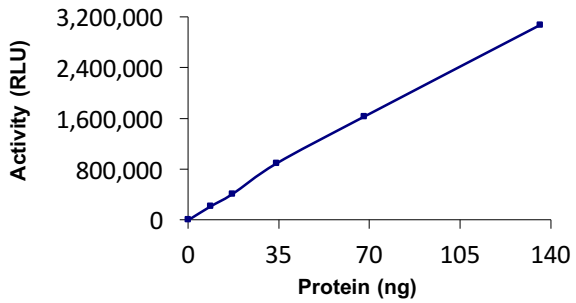
Scientific Background

UBE2A or ubiquitin-conjugating enzyme E2A is a member of the E2 ubiquitin-conjugating enzyme family which is required for post-replicative DNA damage repair and plays an important role in various cellular processes. RAD18 and RAD5 play a central role in mediating physical contacts between the members of the RAD6 pathway. UBE2A is specifically important during chronic low dose ultraviolet exposure to prevent counterproductive DNA checkpoint activation and allow cells to proliferate normally. Loss of the RAD6 error-free PRR pathway results in DNA damage checkpoint-induced G2 arrest in CLUV-exposed cells.

References

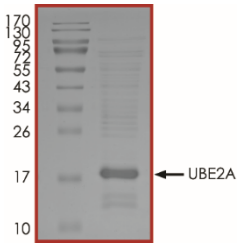
1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. Anal. Biochem. 2016; 510: 41-51
2. Jentsch, S. et al: The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. Nature 1987; 329: 131-134
3. Hishida, T. et al: RAD6-RAD18-RAD5-pathway-dependent tolerance to chronic low-dose ultraviolet light. Nature 2009; 457: 612-615

Specific Activity



The specific activity of UBE2A was determined to be **30 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UBE2A was determined to be **>95%** by densitometry, approx. MW **17 kDa**.

UBE2A, Active

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

Catalog #	U210-380H
Specific Activity	30 nmol/min/mg
Lot #	V2408-7
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 UBE2A (Catalog #:U210-380H) and UBA1 (Catalog #:U201-380G) 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 UBE2A 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 UBE2A 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 UBE2A, UBA1 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 + 50µM ATP
- 2X final concentration of Active UBE2A

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 UBE2A

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)

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