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| Catalog # | Aliquot Size |
|---|---|
| U201-380G-20 | 20 µg |
| U201-380G-50 | 50 µg |

# UBA1 (UBE1), Active

Recombinant full-length human proteins expressed in Sf9 cells

**Catalog # U201-380G**

*Lot # V2408-6*

## Product Description

Full-length recombinant human UBA1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The UBA1 gene accession number is NM_003334.

## Gene Aliases

UBE1, CTD-2522E6.1, A1S9, A1S9T, A1ST, AMCX1, GXP1, POC20, SMAX2, UBA1A, UBE1X

## 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.

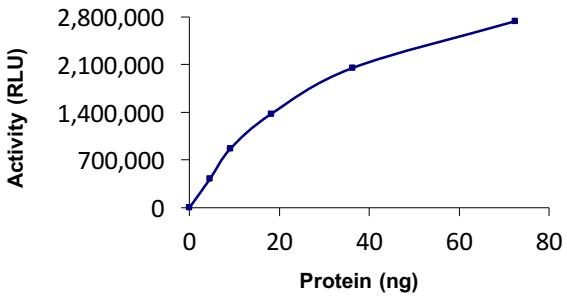
## Scientific Background

Ubiquitin-activating enzyme 1 (UBA1) catalyzes the first step in ubiquitin conjugation to mark cellular proteins for degradation through the ubiquitin-proteasome system. UBA1 activates ubiquitin by first adenylating its C-terminal glycine residue with ATP, and thereafter linking this residue to the side chain of a cysteine residue in E1, yielding a ubiquitin-E1 thioester and free AMP. Posttranslational modification by ubiquitin or ubiquitin-like proteins regulates numerous processes, including cell division, immune responses and embryonic development.

## References

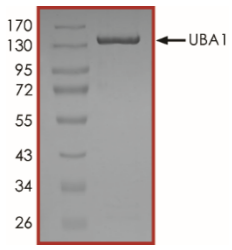

1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. Anal. Biochem. 510: 41-51, 2016
2. Haas, A.L. et al: Ubiquitin-activating enzyme. Mechanism and role in protein-ubiquitin conjugation. J. Biol. Chem. 257(5):2543-2548, 1982
3. McGrath, J.P. et al: UBA 1: an essential yeast gene encoding ubiquitin-activating enzyme. EMBO J. 10(1):227-236, 1991


## Specific Activity




The specific activity of UBA1 was determined to be **110 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of UBA1 was determined to be **>95%** by densitometry, approx. MW **145 kDa**.


# UBA1 (UBE1), Active

Recombinant full-length human protein expressed in Sf9 cells


| | |
|---|---|
| Catalog # | U201-380G |
| Specific Activity | 110 nmol/min/mg |
| Lot # | V2408-6 |
| 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 UBA1 (Catalog #:U201-380G), UBE2C (Catalog #:U212-380H) 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 UBA1 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 UBA1 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 UBA1, UBE2C, 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 + 34ng/µl UBE2C + 60ng/µl BIRC3 + 50µM ATP
- 2X final concentration of Active UBA1

**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 UBA1

*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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