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| Catalog # | Aliquot Size |
|---|---|
| U206-380G-20 | 20 µg |
| U206-380G-50 | 50 µg |

# UBA6, Active

Recombinant full-length human proteins expressed in Sf9 cells

**Catalog # U206-380G**

*Lot # D2542-7*

## Product Description

Recombinant full-length human UBA6 (UBE1L2) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is AL832458.

## Gene Aliases

E1-L2, MOP-4, UBE1L2, DKFZp451P021

## Formulation

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

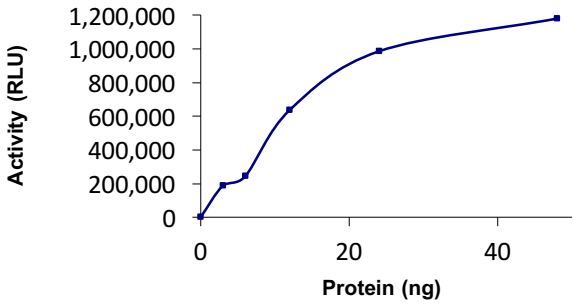
## Scientific Background

UBA6 or ubiquitin-like modifier activating enzyme 6 is an E1 enzyme that initiates the activation and conjugation of ubiquitin-like proteins (1). UBA6 is highly expressed in human tissues and cell lines. E1-L2 activates both ubiquitin and FAT10 (2).

## References

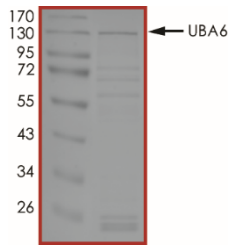

1. Jin, J. et.al: Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. Nature 447: 1135-1138, 2007.
2. Chiu, Y. H. et.al: E1-L2 activates both ubiquitin and FAT10. Molec. Cell 27: 1014-1023, 2007.


## Specific Activity




The specific activity of UBA6 was determined to be **19 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of UBA6 was determined to be **>75%** by densitometry. Approx. MW **146 kDa**.

# UBA6, Active

Recombinant full-length human protein expressed in Sf9 cells

| | |
|---|---|
| Catalog # | U206-380G |
| Specific Activity | 19 nmol/min/mg |
| Lot # | D2542-7 |
| Purity | >75% |
| Concentration | 0.05 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 UBA6 (Catalog #: U206-380G), UBE2D3 (Catalog #: U215-380H) 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 UBA6 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 appropriate working stock.

## Assay Protocol

The UBA6 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin-like (UBL) protein conjugation is proportional to the amount of generated AMP, and the presence of all components of the UBLs conjugation machinery (UBL, E1, E2 and substrate) is required for maximal activity of the system.

**Step 1.** Thaw the active UBA6, UBE2D3, 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 + 10ng/μl UBE2D3 + 42ng/μl BIRC3 + 50μM ATP
- 2X final concentration of Active UBA6

**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 UBA6

*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 30°C for 2 hours

**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)

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