SignalChem
Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
U207-380G-20	20 µg
U207-380G-50	50 µg

UBA7(UBE1L), Active

Recombinant full-length human proteins expressed in Sf9 cells

Catalog # U207-380G

Lot # L2220-10

Product Description

Recombinant full-length human UBA7 (UBE1L) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_003335.

Gene Aliases

D8; UBE2; UBA1B; UBA7; UBE1L

Formulation

Recombinant proteins stored in 50mM Tris-HCl, pH 7.5, 150mM 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.

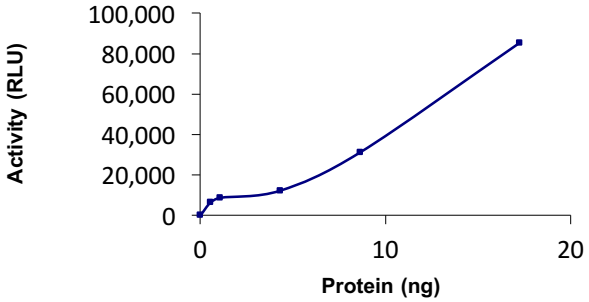
Scientific Background

UBA7 or ubiquitin-like modifier activating enzyme 7 is a member of the E1 ubiquitin-activating enzyme family which is a retinoid target that triggers promyelocytic leukemia (PML)/retinoic acid receptor alpha (RARalpha) degradation and apoptosis in acute promyelocytic leukemia, where it is involved in the conjugation of the ubiquitin-like interferon-stimulated gene 15 protein. UBA7 is expressed in a variety of tissues, including normal lung, but highly expressed in a large series of lung cancer cell lines (1). UBA7 may represent a second, autosomal member of the ubiquitin-activating gene family, which play a role in the ubiquitin conjugation pathway, which is of central importance in all eukaryotes (2).

References

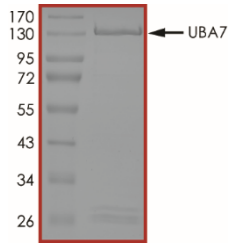
1. Carritt, B. et.al: A gene from human chromosome region 3p21 with reduced expression in small cell lung cancer. Cancer Res. 52: 1536-1541, 1992.
2. Kok, K. et.al: A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitin-activating enzyme. Proc. Nat. Acad. Sci. 90: 6071-6075, 1993.

Specific Activity



The specific activity of UBA7 was determined to be **50 nmol /min/mg** as per activity assay protocol.

Purity



The purity of UBA7 was determined to be **>95%** by densitometry. Approx. MW **138kDa**.

UBA7 (UBE1L), Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	U207-380G
Specific Activity	50 nmol/min/mg
Lot #	*L2220-10*
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

Activity Assay Protocol

Reaction Components

Active Enzymes

Active UBA7 (Catalog #: U207-380G) and UBE2L6 (Catalog #: U229-380H) diluted with SUMOylation 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 UBA7 for optimal results).

SUMOylation Buffer

Buffer components: 50mM Tris-HCl (pH7.5), 5mM $MgCl_2$. 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

Human ISG15 protein diluted with SUMOylation Buffer to appropriate working stock.

Assay Protocol

The UBA7 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 UBA7, UBE2L6 and ISG15 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 SUMOylation Buffer:

- 2X Reaction Cocktail: 380ng/µl ISG15 + 7ng/µl UBE2L6 + 50µM ATP
- 2X final concentration of Active UBA7

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 UBA7

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of SUMOylation 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)

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