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| Catalog # | Aliquot Size |
|---|---|
| U213-380H-20 | 20 µg |
| U213-380H-50 | 50 µg |

# UBE2D1 (UBCH5A), Active

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

**Catalog # U213-380H**

*Lot # V2408-9*

## Product Description

Recombinant full-length human UBE2D1 was expressed in *E. coli* cells using an N-terminal His tag. The UBE2D1 gene accession number is NM_003338.

## Gene Aliases

E2(17)KB1, SFT, UBC4/5, UBCH5, UBCH5A

## 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.

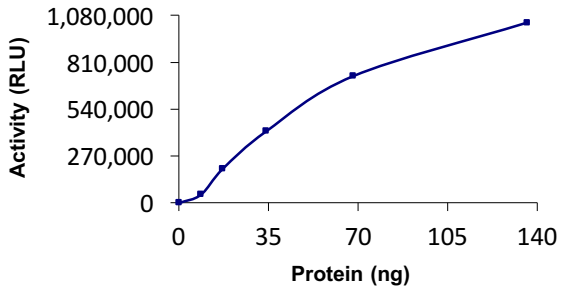
## Scientific Background

UBE2D1 (UBCH5A) or ubiquitin-conjugating enzyme E2D1 is a member of the E2 ubiquitin-conjugating enzyme family which is closely related to a stimulator of iron transport (SFT). UBE2D1 is significantly upregulated in livers of iron-overloaded patients with hereditary hemochromatosis. UBE2D1 also functions in the ubiquitination of the tumor-suppressor protein p53 and the hypoxia-inducible transcription factor HIF-1α by interacting with the E1 ubiquitin-activating enzyme and the E3 ubiquitin-protein ligases.

## References

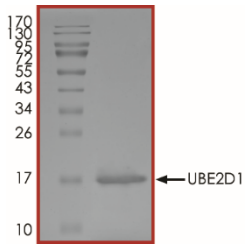

1. Mondal, S. et al: A bioluminescent assay for monitoring conjugation of ubiquitin and ubiquitin-like proteins. Anal. Biochem. 510: 41-51, 2016
2. Gehrke, S.G. et al: UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. Blood 101: 3288-3293, 2003
3. Scheffner, M. et al: Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. Proc. Nat. Acad. Sci. 91: 8797-8801, 1994


## Specific Activity




The specific activity of UBE2D1 was determined to be **22 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of UBE2D1 was determined to be **>95%** by densitometry, approx. MW **17 kDa**.


# UBE2D1 (UBCH5A), Active

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


| | |
|---|---|
| Catalog # | U213-380H |
| Specific Activity | 22 nmol/min/mg |
| Lot # | V2408-9 |
| 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 UBE2D1 (Catalog #:U213-380H), 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 UBE2D1 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 UBE2D1 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 UBE2D1, 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 UBE2D1

**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 UBE2D1

*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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