

Certificate of Analysis

UBE1, active

(Recombinant UBE1 expressed in Sf21 insect cells)

Item # 23-021, 23-021-K, 23-021M

Parent Lot # D11CP024N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant human UBE1 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 122kDa.

Formulation: 2.484mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Activity (Parent lot# D11CP024N):

This lot of UBE1, active is active and meets product specifications.

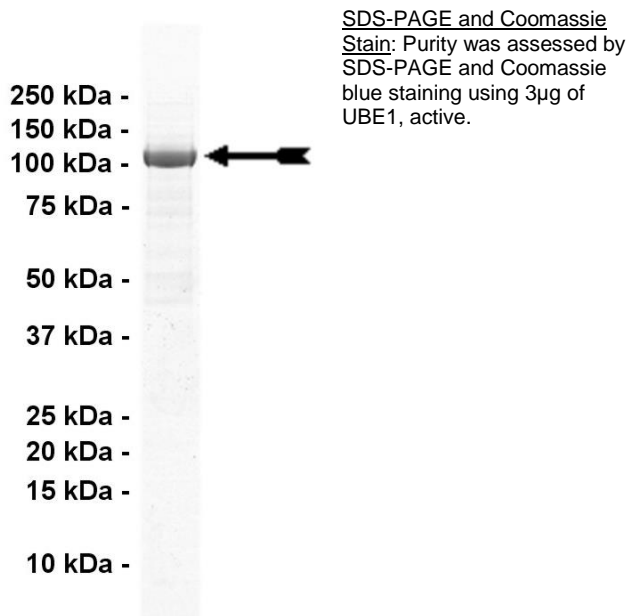
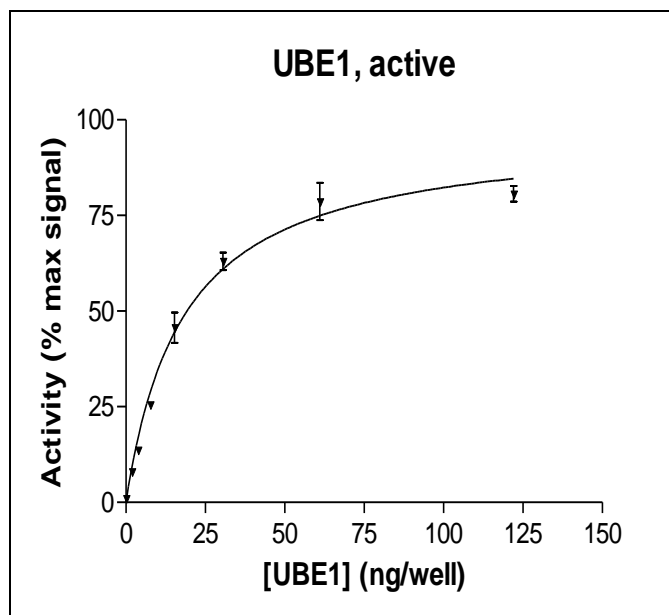
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

Protein Identity: Confirmed identity as UBE1 by mass spectrometry.



Certificate of Analysis

UBE1 Assay Protocol

Reagents:

- | | |
|--------------------------------|----------------------------------|
| 1. UBE1 (Item # 23-021) | 3. Biotinylated-Ubiquitin |
| 2. 1x Reaction Buffer | 4. Stop Solution |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl₂).

UBE1 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-6His antibody and washing with PBS containing 0.05% Tween 20. UBE1 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

Certificate of Analysis

UBE1 Information

<u>Protein</u>	Human, UBE1
<u>Accession number</u>	GenBank NM_003334
<u>Alternate Names</u>	Ubiquitin activating enzyme E1, Ubiquitin-like modifier-activating enzyme 1, UBA1, A1S9 Protein

<u>Key Facts</u>	UBE1 catalyzes the first step in ubiquitin conjugation to mark cellular proteins for degradation. It is responsible for the primary ubiquitin-protein isopeptide bond formation and is a critical component for the initiation of <i>in vitro</i> conjugation reactions. UBE1 activates ubiquitin by the ATP dependent adenylation of its C-terminal glycine carboxyl group. This ubiquitin residue is then linked to the sulphhydryl side chain moiety of a cysteine residue within UBE1 by forming a high energy thiol ester bond, liberating free AMP. There are two active sites within the UBE1 molecule allowing it to accommodate two ubiquitin molecules at one time, with a new ubiquitin forming an adenylate intermediate as the previous one is transferred to the thiol site. The activated ubiquitin is then transferred to the lysine of target proteins via the E2/E3 conjugation cascade.
-------------------------	--

<u>Related Products</u>	Item# 23-022 UbchH3, active, Item# 23-025 UbchH4, active, Item# 23-029 UbchH5a, active
--------------------------------	--

Selected References

- Haas A.L. and Rose I.A. The Mechanism of Ubiquitin Activating Enzyme. A Kinetic and Equilibrium Analysis. J Biol Chem., 257: 10329-10337, 1982
- Haas A.L., *et al.* Ubiquitin-activating Enzyme. Mechanism and Role in protein Ubiquitin Conjugation. J Biol Chem., 257: 2543-2548, 1982
- Handley P.M., *et al.* Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1. Proc. Natl. Acad. Sci. USA., 88: 258-262, 1991
- Lee I, and Schindelin H., Structural Insights into E1-Catalyzed Ubiquitin Activation and Transfer to Conjugating Enzymes. Cell, 134: 268–278, 2008

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services