

Certificate of Analysis

Ubch5a, active

(Recombinant ubiquitin conjugating enzyme (E2) expressed in *E.coli*)

Item # 23-029, 23-029-K, 23-029M

Parent Lot # D11EP014N

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 HA, 6His-tagged, recombinant human Ubch5a full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Formulation: 4.507mg/ml of enzyme in 50mM Tris/HCl pH8.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 19kDa.

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# D11EP014N): This lot of Ubch5a is active and meets product specifications.

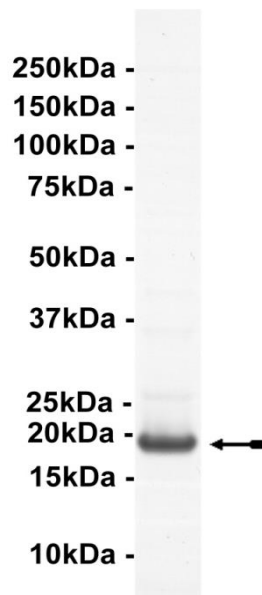
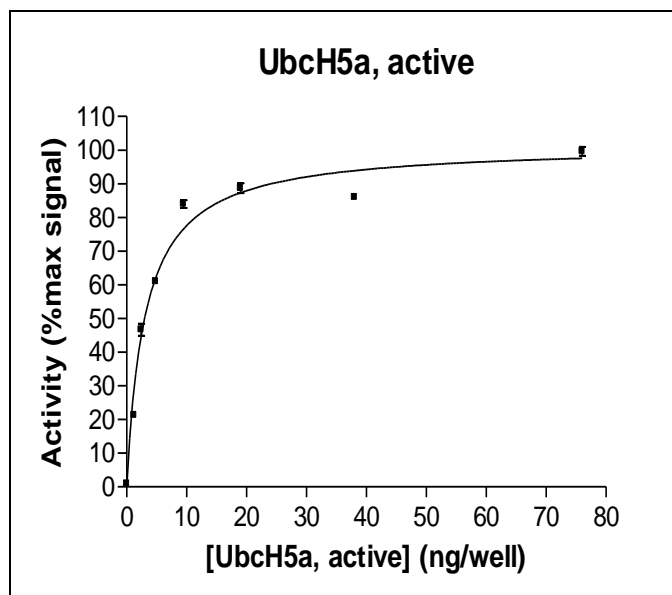
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 Ubch5a by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Ubch5a, active.

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E2 Assay Protocol

Reagents:

- | | |
|-----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021) | 4. Biotinylated-Ubiquitin |
| 2. Ubch5a, active (Item # 23-029) | 5. Stop Solution |
| 3. 1x Reaction Buffer | |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂).

Ubch5a is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, 10nM UBE1, 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-HA antibody and washing with PBS containing 0.05% Tween 20. Ubch5a activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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Ubch5a Information

<u>Protein</u>	human Ubch5a
<u>Accession number</u>	GenBank NM_003338
<u>Alternative Names</u>	Ubiquitin-conjugating enzyme E2 D1, Ubiquitin-protein ligase D1, Ubiquitin-conjugating enzyme E2-17 kDa 1, UBC4/5 homolog, Stimulator of Fe transport (SFT), Ubch5, UBE2D1
<u>Key Facts</u>	<p>Ubiquitin-conjugating (E2) enzymes are characterized by the presence of a highly conserved ubiquitin-conjugating domain which accommodates ATP-activated ubiquitin (Ub) via a covalently linked thioester onto its active-site residue. E2 enzymes act via selective protein-protein interactions with the ubiquitin-activating E1 enzyme and ubiquitin ligase E3 enzymes and are able to differentiate effects on downstream substrates, either with a single Ub molecule or an Ub chain. While E3s are involved in substrate selection, E2s are the main determinants for selection of the lysine to construct Ub chains, which thereby directly control the cellular fate of the substrate.</p> <p>The Ubch5 family (Ubch5a, Ubch5b and Ubch5c) have been shown to be the most active class of E2 enzymes in cell extracts and are associated with the regulation of a number of transcription factors, including p53 and c-Myc.</p>
<u>Related Products</u>	Item # 23-021 UBE1, active, Item # 23-030 SCF ^{Fbw7} complex, active

Selected References

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Wenzel D.M. *et al.* E2s: Structurally Economical and Functionally Replete. *Biochem. J.*, 443: 31-42, 2011

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Brzovic P.S. and Klevit R.E. Ubiquitin Transfer from the E2 Perspective. Why is Ubch5 so Promiscuous? *Cell Cycle* 5: 2867-2873, 2006

Scheffner M. *et al.*, Identification of a Human Ubiquitin-Conjugating Enzyme that Mediates the E6-AP-Dependent Ubiquitination of p53. *PNAS*, 91: 8797-8801, 1994

Yada M. *et al.*, Phosphorylation-Dependent Degradation of c-Myc is Mediated by the F-box Protein Fbw7. *The EMBO Journal* 23: 2166-2125, 2004

Reviewed and approved by site quality representative.

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