

## Certificate of Analysis

### Ubch5c, active

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

Item # 23-035, 23-035-K, 23-035M

Parent Lot # D11DP024N

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 Ubch5c full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 19kDa.

#### Activity (Parent lot# D11DP024N):

This lot of Ubch5c is active and meets product specifications.

**Formulation:** 3.332mg/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.

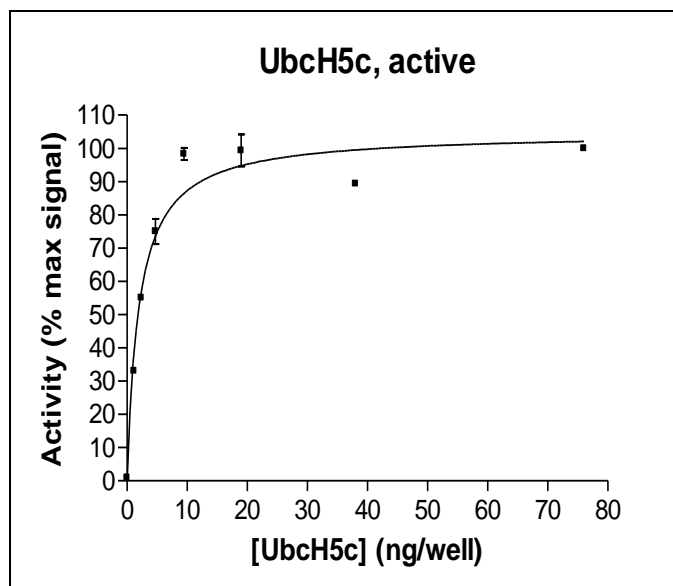
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 Ubch5c by mass spectrometry.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Ubch5c, active.

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### E2 Assay Protocol

#### Reagents:

- |                                   |                           |
|-----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021)   | 4. Biotinylated-Ubiquitin |
| 2. UbcH5c, active (Item # 23-035) | 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 MgCl<sub>2</sub>).

UbcH5c is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 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 pH 7.5 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. UbcH5c activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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### Ubch5c Information

<b><u>Protein</u></b>	human Ubch5c
<b><u>Accession number</u></b>	GenBank NM_181888
<b><u>Alternative Names</u></b>	Ubiquitin-conjugating enzyme E2 D3, Ubiquitin-protein ligase D3, Ubiquitin-conjugating enzyme E2-17 kDa 3, UBE2D3
<b><u>Key Facts</u></b>	<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 a 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 control of a number of proteins including the transcriptional regulators p53, IκBα and HIF-1α.</p>
<b><u>Related Products</u></b>	Item # 23-021 UBE1, active, Item # 23-032 MDM2/CK1δ, active, Item # 23-033 MDM2 (c-Myc tagged), active

### **Selected References**

- Sadowski M. and Sarcevic B. Mechanisms of Mono- and Poly-Ubiquitination: ubiquitination specificity depends on compatibility between the E2 catalytic core and amino acid residues proximal to the lysine. *Cell Division*, 5: 19, 2010
- Wenzel D.M. *et al.* E2s: Structurally Economical and Functionally Replete. *Biochem. J.*, 443: 31-42, 2011
- van Wijk S. J. L and Timmers H. T. M. The Family of Ubiquitin-Conjugating Enzymes (E2s):deciding between life and death of proteins. *The FASEB Journal*, 24: 981-993, 2010
- Saville M.K. *et al.*, Regulation of p53 by the Ubiquitin-Conjugating Enzymes Ubch5B/C *in vivo*. *J Biol Chem*, 279: 42169-42181, 2004
- Gonen H. *et al.*, Identification of the Ubiquitin Carrier Proteins, E2s, Involved in Signal-Induced Conjugation and Subsequent Degradation of IκappaBα. *J Biol Chem*. 274: 14823-14830, 1999

Reviewed and approved by site quality representative.

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