

Certificate of Analysis

Ubch6, active

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

Item # 23-036, 23-036-K, 23-036M

Parent Lot # D11DP023N

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 Ubch6 full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Purity 95.9% by SDS-PAGE and Coomassie blue staining. MW = 24kDa.

Activity (Parent lot# D11DP023N): This lot of Ubch6 is active and meets product specifications.

Formulation: 2.491mg/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.

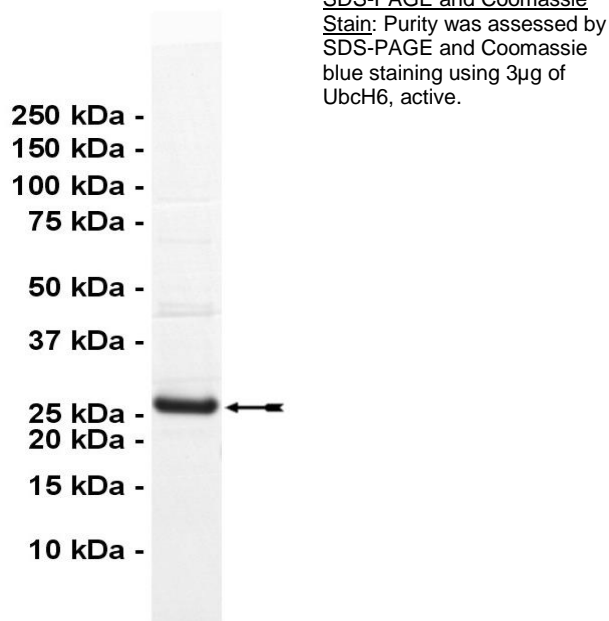
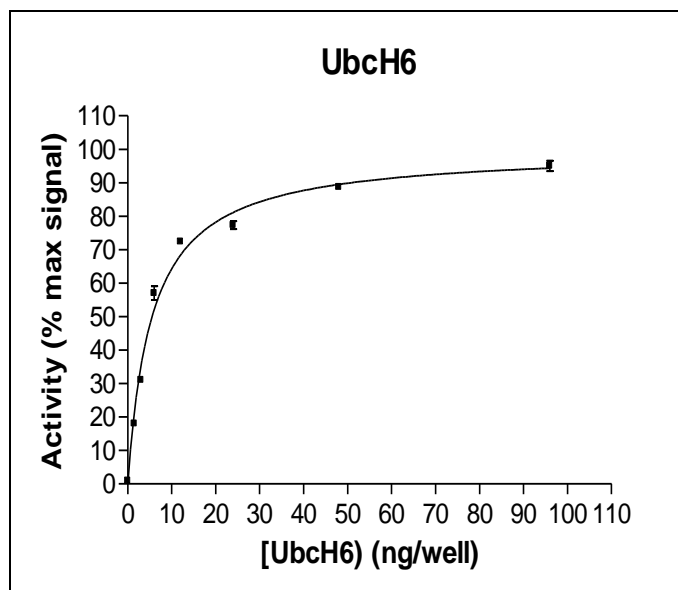
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 Ubch6 by mass spectrometry.



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E2 Assay Protocol

Reagents:

- | | |
|----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021) | 4. Biotinylated-Ubiquitin |
| 2. UbcH6, active (Item # 23-036) | 5. Stop Solution |
| 3. 1x Reaction Buffer | |

Assay Outline:

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

UbcH6, active 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 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. UbcH6 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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UbchH6 Information

Protein human UbchH6

Accession number GenBank NM_003341

Alternative Names Ubiquitin-conjugating enzyme E2 E1, Ubiquitin-protein ligase E1, UBE2E1

Key Facts 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. UbchH6 has been shown to interact with a number of diverse E3 ligases, including RNF20/40, Trim32 and WWP2.

Related Products Item # 23-021 UBE1, active, Item # 23-048 Parkin, active, Item # 23-046 Parkin (c-Myc tagged), active, Item # 23-049 p38/JTV-1

Selected References

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Reviewed and approved by site quality representative.

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