

Certificate of Analysis

UbchH3, active

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

Item # 23-022, 23-022-K, 23-022M

Parent Lot # D11EP005N

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 UbchH3 full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Purity 84.2% by SDS-PAGE and Coomassie blue staining. MW = 29kDa.

Activity (Parent lot# D11EP005N): This lot of UbchH3 is active and meets product specifications.

Formulation: 2.728mg/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.

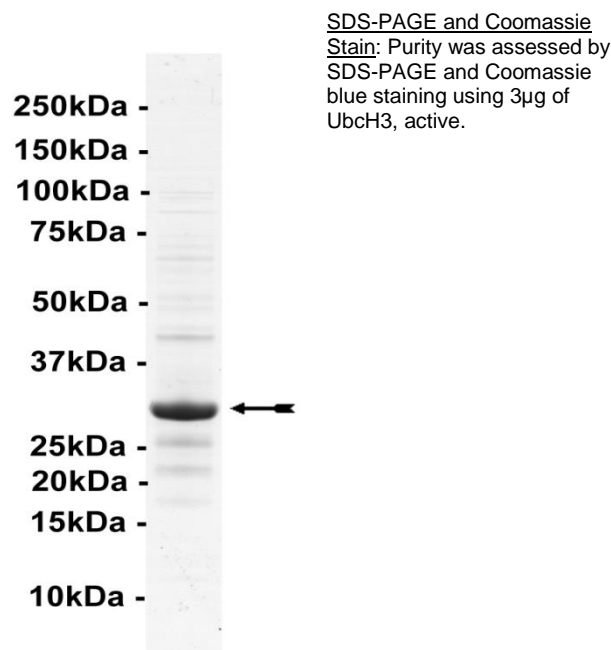
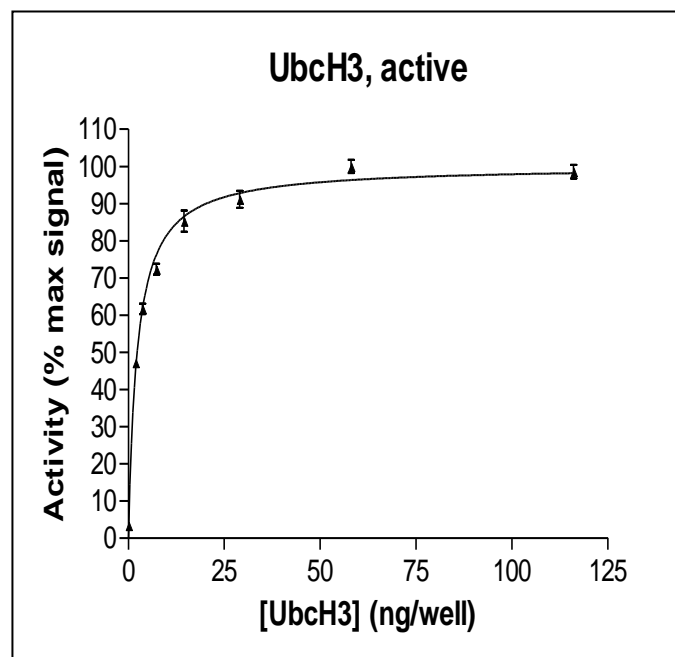
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 UbchH3 by mass spectrometry.



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E2 Assay Protocol

Reagents:

- | | |
|---|----------------------------------|
| 1. UBE1, active (Item # 23-021) | 4. Biotinylated-Ubiquitin |
| 2. UbcH3, active (Item # 23-022) | 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).

UbcH3 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. UbcH3 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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Ubch3 Information

Protein human Ubch3

Accession number GenBank NM_004359

Alternative Names Ubiquitin-conjugating enzyme E2 R1, Ubiquitin-protein ligase R1, CDC34, **UBE2R1**, E2-CDC34

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 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. Ubch3 has been shown to interact with a number of different E3 ligases, including SCF^{Skp2/Cks1}, promoting the ubiquitination of p27 and SCF^{βTrCP1}, promoting the ubiquitination of β-catenin and IκBa.

Related Products Item # 23-021 UBE1, active, Item # 23-023 SCF^{Skp2/Cks1} complex, active, Item # 23-026 SCF^{βTrCP1} complex, active, Item# 23-030 SCF^{Fbw7} complex, active

Selected References

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Xu S. *et al.* Substrate Recognition and Ubiquitination of SCF^{Skp2/Cks1} Ubiquitin-Protein Isopeptide Ligase. J Biol Chem. 282: 15462-15470, 2007

Strack P. *et al.* SCF^{β-TRCP} and Phosphorylation Dependent Ubiquitination of IκBa Catalyzed by Ubc3 and Ubc4. Oncogene, 19: 3529-3536, 2000

Reviewed and approved by site quality representative.

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