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Certificate of Analysis

UbcH7, active

(Recombinant ubiquitin conjugating enzyme (E2) expressed in *E.coli*)
Item # 23-047, 23-047-K, 23-047M
Parent Lot # D11BP012N

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 UbcH7 full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.
Purity 90% by SDS-PAGE and Coomassie blue staining. MW = 20kDa.

Activity (Parent lot# D11BP012N): This lot of UbcH7 is active and meets product specifications.

Formulation: 3.264mg/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.

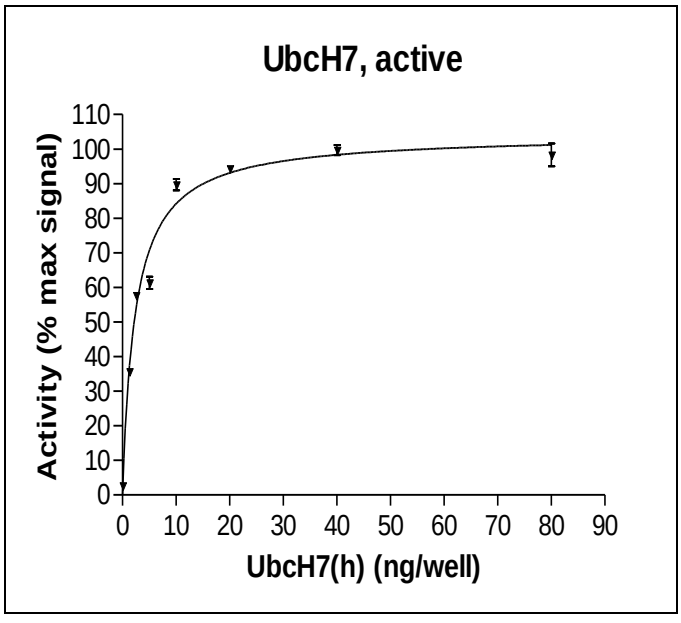
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

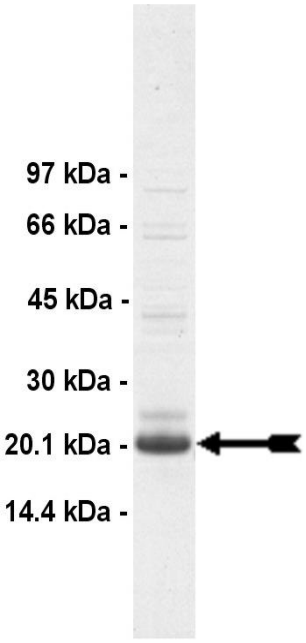
Quality Control Testing

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



Protein Identity: Confirmed identity as UbcH7 by mass spectrometry.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of UbcH7, active.



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E2 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH7, active (Item # 23-047)**
3. **1x Reaction Buffer**
4. **Biotinylated-Ubiquitin**
5. **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 $MgCl_2$).

UbcH7 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 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. UbcH7 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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UbcH7 Information

Protein human UbcH7

Accession number GenBank X92962

Alternative Names Ubiquitin-conjugating enzyme E2 L3, L-UBC, Ubiquitin carrier protein L3, Ubiquitin-conjugating enzyme E2-F1, Ubiquitin-protein ligase L3, UBE2L3, UBCE7

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.
UbcH7 shows broad specificity for HECT-type E3s but does not function with most RING-containing E3 ubiquitin-protein ligases because it lacks intrinsic E3-independent reactivity with lysine. Despite lacking this lysine reactivity, UbcH7 exhibits activity with the RBR family E3 enzymes, such as Parkin and ARIH1.

Related Products Item # 23-021 UBE1, active, Item # 23-046 Parkin (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

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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

Wenzel D. M. *et al*, UbcH7 Reactivity Profile Reveals Parkin and HHARI to be RING/HECT Hybrids. Nature, *474*: 105-108, 2011

Schwarz S. E. *et al*, Characterization of Human Hect Domain Family Members and their Interaction with UbcH5 and UbcH7. J Biol Chem, *273:* 12148-12154, 1998

Imai Y. *et al*, Parkin Suppresses Unfolded Protein Stress-Induced Cell Death Through its E3 Ubiquitin-Protein Ligase Activity. J Biol Chem, *275:* 35661-35664, 2000

Reviewed and approved by site quality representative.