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Certificate of Analysis

UbcH4, active

(Recombinant ubiquitin conjugating enzyme (E2) expressed in *E.coli*)
Item # 23-025, 23-025-K, 23-025M
Parent Lot # D11EP004N

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 UbcH4 full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.

Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 19kDa.

Activity (Parent lot# D11EP004N): This lot of UbcH4 is active and meets product specifications.

Formulation: 5.491mg/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

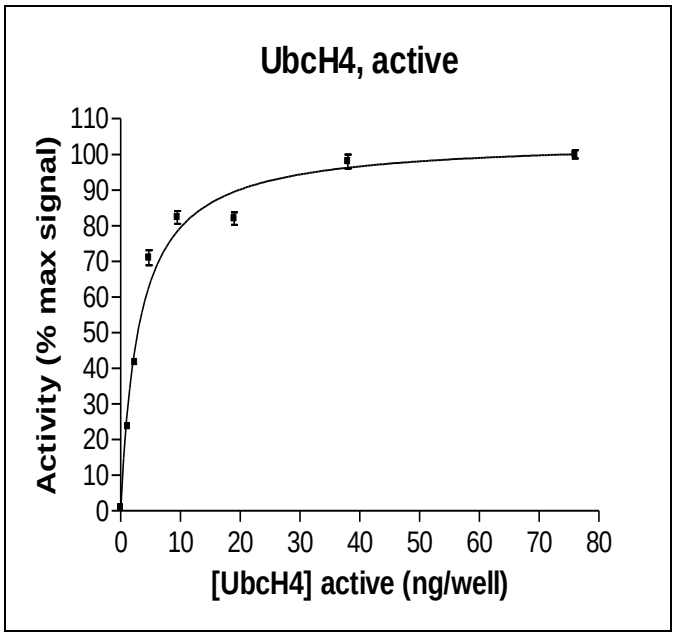
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

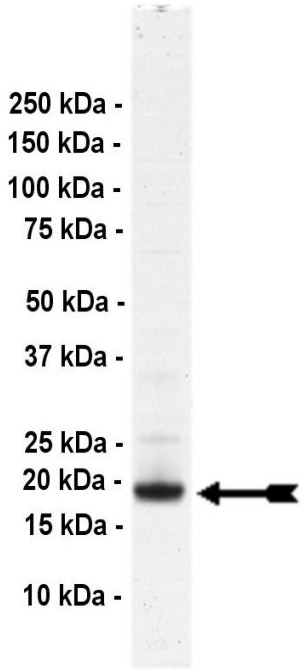
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 UbcH4 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active UbcH4.

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E2 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH4, active (Item # 23-025)**
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 $MgCl_2$).

UbcH4 is incubated with 25mM MOPS pH 7.5, 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 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. UbcH4 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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UbcH4 Information

Protein human UbcH4

Accession number GenBank NM_003339

Alternative Names Ubiquitin-conjugating enzyme E2 D2, Ubiquitin-protein ligase D2, UbcH5b, Ubiquitin-conjugating enzyme E2-17kDa 2, UBE2D2

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.
The UbcH5 family (UbcH5a, UbcH5b/UbcH4 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 proteins associated with cell signalling, including p53, IκBα and β-catenin.

Related Products Item # 23-021 UBE1, active, Item # 23-026 $SCF^{\beta TrCP1}$ complex, 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 IkappaBalpha. J Biol Chem. *274*: 14823-14830, 1999

Strack P. *et al*., $SCF^{b\text{-}TRCP}$ and Phosphorylation Dependent Ubiquitination of IκBα Catalysed by Ubc3 and Ubc4. Oncogene, *19*: 3529-3536, 2000

Reviewed and approved by site quality representative.