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# Certificate of Analysis

## UbcH13/Uev1A, active

**(Recombinant ubiquitin conjugating enzyme (E2) expressed in *E.coli*)**
**Item # 23-051, 23-051-K, 23-051M**
**Parent Lot # D11CP007N**

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:** Complex of *N*-terminal HA, 6His-tagged, recombinant human UbcH13 full length and untagged recombinant human Uev1A full length, expressed in *E.coli*. Purified using immobilized metal affinity chromatography.
Purity 99% by SDS-PAGE and Coomassie blue staining. UbcH13 MW = 19kDa, Uev1A MW = 17kDa

**Activity (Parent lot# D11CP007N):** This lot of UbcH13/Uev1A is active and meets product specifications.

**Formulation: 3.084mg/ml** of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

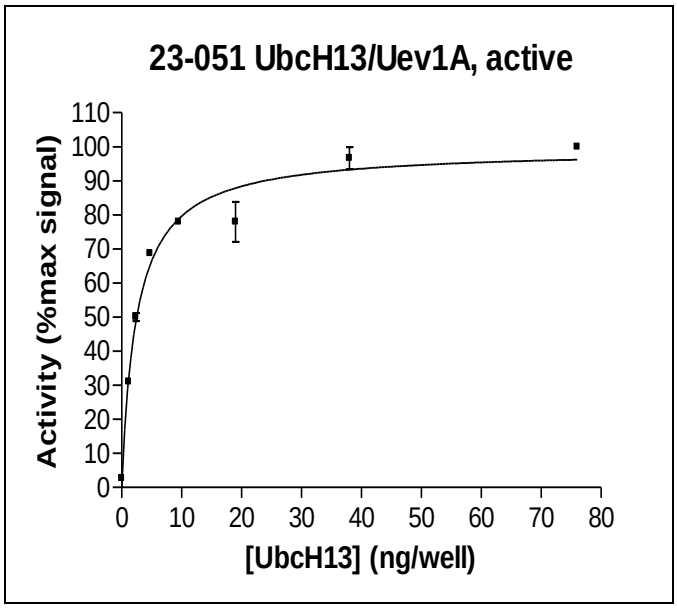
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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**

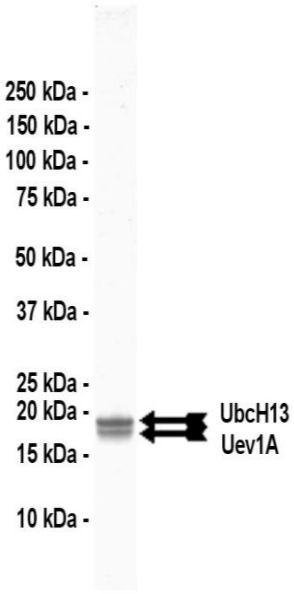
## Quality Control Testing

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




Protein Identity: Confirmed identity as UbcH13 and Uev1A by mass spectrometry.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of UbcH13/Uev1A, active.

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# Certificate of Analysis

## E2 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH13/Uev1A, active (Item # 23-051)**
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 $MgCl_2$).

UbcH13/Uev1A 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. UbcH13/Uev1A activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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## UbcH13/Uev1A Information

**Protein** human UbcH13, human Uev1A

**Accession number** GenBank NM_003348 UbcH13, GenBank NM_001032288 Uev1A

**Alternative Names** UbcH13 - Ubiquitin-conjugating enzyme E2 N, Bendless-like ubiquitin-conjugating enzyme, Ubiquitin carrier protein N, UBE2N, Ubc13
Uev1A - Ubiquitin-conjugating enzyme E2 variant 1, CROC-1, UBE2V1

**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. Typically, a polyubiquitin chain that targets a protein for degradation by the proteasome is linked through lysine-48 (K48) of ubiquitin, however ubiquitin chains linked through other lysines such as K63 have also been found. K63 linked chains are non-degradative and play a role in signalling and protein trafficking.
The heterodimeric E2 complex of Ubc13 and Uev1A has been shown to be involved in the formation of K63 linkages in conjunction with a number of E3 ligases including Parkin, CHIP and TRAF6.

**Related Products** Item # 23-021 UBE1, active, Item # 23-053 CHIP (c-Myc tagged), active, Item # 23-050 CHIP, active, Item # 23-034 p53, Item # 23-052 Hsp70

**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

Windheim M. *et al.,* Two Different Classes of E2 Ubiquitin-Conjugating Enzymes are Required for the Mono-Ubiquitination of Proteins and Elongation by Polyubiquitin Chains with a Specific Topology. Biochem J., *409*: 723-729, 2008

Petroski M. D*. et al*., Substrate Modification with Lysine 63-Linked Ubiquitin Chains Through the UBC13-UEV1A Ubiquitin-Conjugating Enzyme. J Biol Chem., *282*: 29936-29945, 2007


Reviewed and approved by site quality representative.