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Certificate of Analysis

MDM2/CK1δ, active

(Recombinant E3 ligase expressed in *E.coli*)
Item # 23-032, 23-032-K, 23-032M
Parent Lot # D11HP018N

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 GST-tagged, recombinant human MDM2 full length, expressed in *E.coli*. Purified using glutathione sepharose followed by gel filtration. Combined *in vitro* with CK1δ, active (Item # 14-520)

Combined Purity 78% by SDS-PAGE and Coomassie blue staining. MDM2 MW = 82kDa, CK1δ MW = 61kDa.

Activity (Parent lot# D11HP018N): This lot of MDM2/CK1δ is active and meets product specifications.

Formulation: 0.100mg/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.

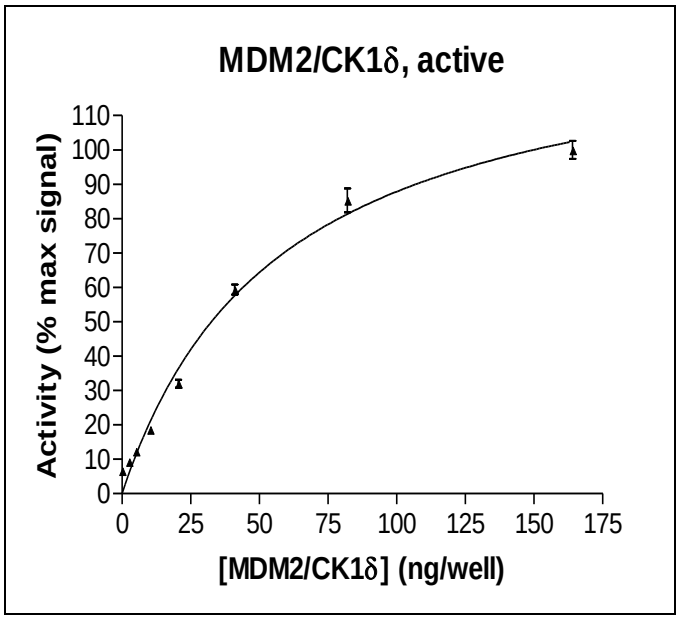
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

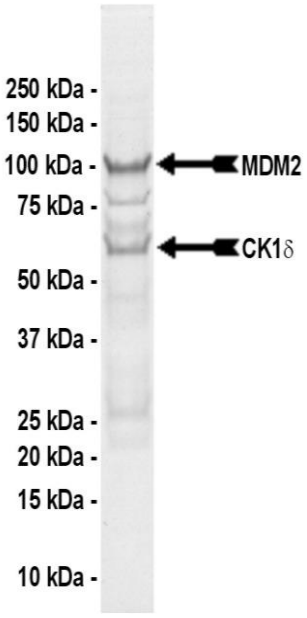
Quality Control Testing

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



Protein Identity: Confirmed identity as MDM2 and CK1δ by mass spectrometry.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MDM2/CK1δ, active.



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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH4, active (Item # 23-025)**
3. **MDM2/CK1δ, active (Item # 23-032)**
4. **p53 (Item # 23-034)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **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 $MgCl_2$).

MDM2/CK1δ, active, is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10μM ATP, 10nM UBE1, 500nM UbcH4, 125nM p53, 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-c-Myc antibody and washing with PBS containing 0.05% Tween 20. MDM2/CK1δ, activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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MDM2 Information

Protein human MDM2

Accession number GenBank AF527840

Alternative Names Double minute 2 protein, p53-binding protein Mdm2, Hdm2

Key Facts MDM2 is an E3 ubiquitin ligase that regulates the turnover of several cellular factors including the p53 tumour suppressor protein and notably MDM2 itself. It contains a number of functional elements including an *N*-terminal p53 binding domain, a *C*-terminal RING finger domain that mediates ubiquitin transfer, and a central acidic region critical for MDM2 function. Several human tumour types have been shown to have increased levels of MDM2, including soft tissue sarcomas, osteosarcomas and breast tumours.

Related Products Item # 23-021 UBE1, active, Item # 23-025 UbcH4, active, Item # 23-035 UbcH5c, active, Item # 23-034 p53

Selected References

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Honda R. *et al.,* Oncoprotein MDM2 is a Ubiquitin Ligase E3 for Tumor Suppressor p53. FEBS Lett. *420*: 25-27, 1997

Marine J-C. and Lozano G. Mdm2-Mediated Ubiquitylation: p53 and Beyond. Cell Death and Differentiation, *17*: 93-102, 2010

Huart A-S. *et al*., CK1α Plays a Central Role in Mediating MDM2 Control of p53 and E2F-1 Protein Stability. J. Biol Chem., *284*: 32384-32394, 2009

Sigalas I. *et al*., Alternatively Spliced Mdm2 Transcripts with Loss of p53 Binding Domain Sequences: Transforming Ability and Frequent Detection in Human Cancer. Nat Med. *2*: 912-917, 1996

Reviewed and approved by site quality representative.