

## Certificate of Analysis

**MDM2 (c-Myc tagged), active**  
 (Recombinant E3 ligase expressed in *E.coli*)  
 Item # 23-033, 23-033-K, 23-033M  
 Parent Lot # D11HP016N

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 c-Myc, GST-tagged, recombinant human MDM2 full length, expressed in *E.coli*. Purified using glutathione sepharose followed by gel filtration.

Purity 51% by SDS-PAGE and Coomassie blue staining. MW = 83kDa.

**Activity (Parent lot# D11HP016N):**  
 This lot of MDM2 (c-Myc tagged) is active and meets product specifications.

**Formulation:** 0.364mg/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.

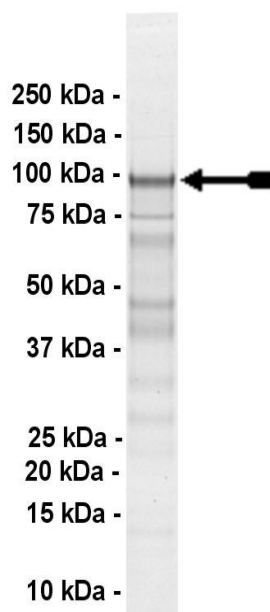
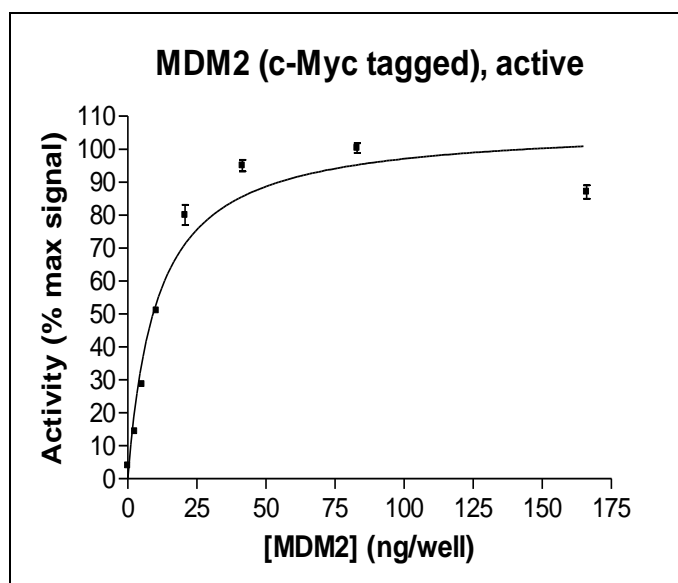
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 by mass spectrometry.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of MDM2 (c-Myc tagged), active.

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### E3 Assay Protocol

#### Reagents:

- |  |                           |
|--|---------------------------|
| 1. UBE1, active (Item # 23-021)                | 4. 1x Reaction Buffer     |
| 2. Ubch4, active (Item # 23-025)               | 5. Biotinylated-Ubiquitin |
| 3. MDM2 (c-Myc tagged), active (Item # 23-033) | 6. 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<sub>2</sub>).

MDM2 (c-Myc tagged), active is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 10μM ATP, 10nM UBE1, 500nM Ubch4 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-c-Myc antibody and washing with PBS containing 0.05% Tween 20. MDM2 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 UbchH4, active, Item # 23-035 UbchH5c, active

### **Selected References**

Fang S. *et al.*, Mdm2 is a RING Finger-Dependent Ubiquitin Protein Ligase for Itself and p53. *J Biol Chem.*, 275: 8945-8951, 2000

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

Huatt A-S. *et al.*, CK1 $\alpha$  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.

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