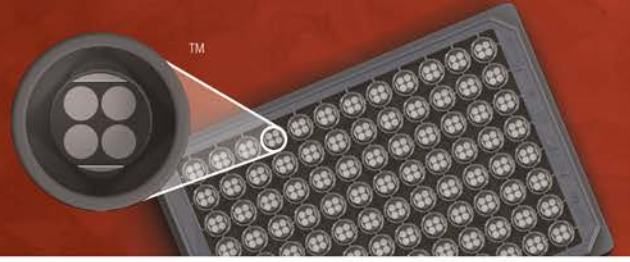
MSD® Ubiquitinated/Total MDM2 Assay Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Ubiquitinated/Total MDM2 Whole Cell Lysate Kit	
Kit size	
1 plate	K15168D-1
5 plates	K15168D-2
20 plates	K15168D-3

Ubiquitinated MDM2 Whole Cell Lysate Set	
200 µg	C12FJ-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
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Email: CustomerService@mesoscale.com

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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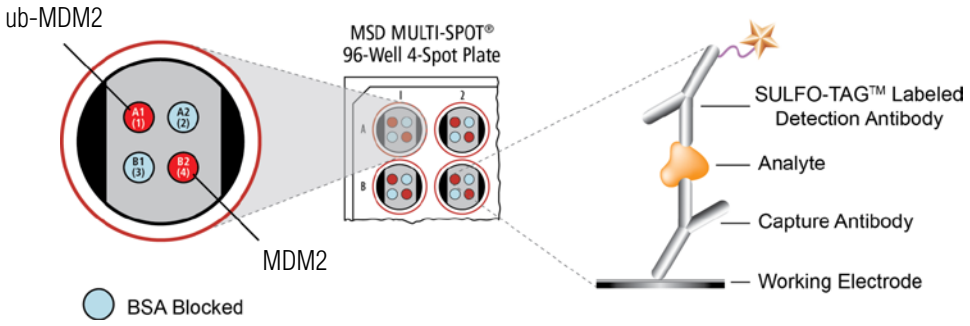

MDM2 (Murine Double Minute 2), an E3 ubiquitin ligase and a negative regulator of p53, is a 56 kDa oncoprotein which is ubiquitinated and phosphorylated. MDM2 contains an amino terminal p53 interaction domain, an acidic domain in the region of amino acids 250-300 (phosphorylation within this region is believed to play a role in MDM2 regulation), and a carboxy-terminal RING domain containing a Cis2-His2-Cis4 consensus motif which binds zinc and is responsible for the E3 ubiquitin ligase activity of MDM2.[1] MDM2 degradation is controlled by self-ubiquitination, phosphorylation, and potentially through ubiquitination by other not yet identified E3 ligases.[2] DNA damage and cellular stress trigger MDM2 degradation, releasing p53 from MDM2 mediated negative regulation.[3] Deletion of MDM2 in mouse models is lethal in a p53 dependent manner,[4] and overexpression of MDM2 is seen in many cancers with non-mutated p53, leading to the conclusion that MDM2 is oncogenic by way of p53 inactivation.[5] Due to the important role p53 tumor suppression plays in many different forms of cancer, there has been extensive research on the interactions between MDM2 and p53 and considerable interest in identifying drugs capable of modulating the MDM2 - p53 interaction.

The MSD Ubiquitinated/Total MDM2 Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Ubiquitinated/Total MDM2 Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with a total MDM2 antibody and are shown below for comparison. Growing HCT116 cells (negative) were treated with doxorubicin (1 µM; 21 hours) and epoxomicin (1 µM; 6 hours) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with antibody against ubiquitinated proteins and anti-total MDM2 antibody on spatially distinct electrodes within a well. Ubiquitinated and total MDM2 were detected with anti-total MDM2 antibody conjugated with MSD SULFO-TAG™ reagent.

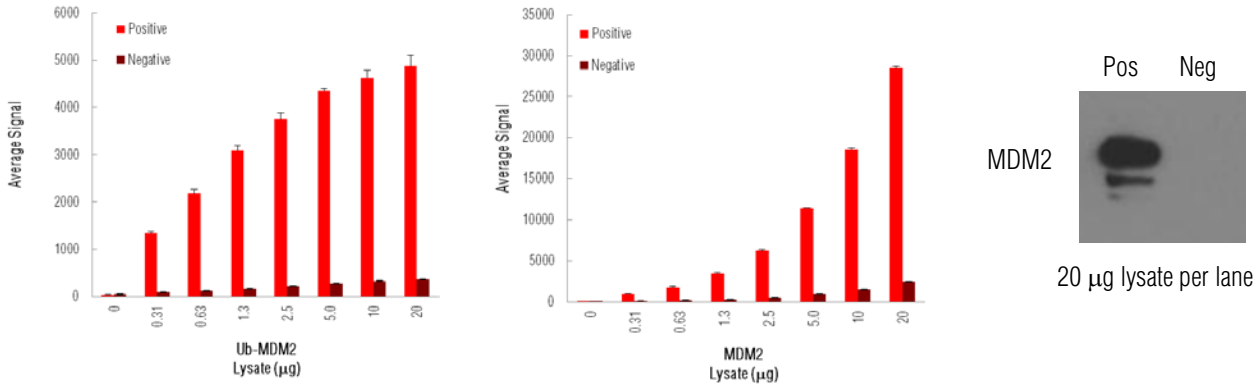

Fig. 1: Sample data generated with MULTI-SPOT Ubiquitinated/Total MDM2 Assay. Increased signal was observed with the titration of MDM2 positive cell lysate. Signal for the negative lysate remains low throughout the titration. The Ubiquitinated/Total MDM2 Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative HCT116 cell lysates using the MULTI-SPOT Ubiquitinated/Total MDM2 Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
ub-MDM2	0	34	3	8.8	40	1	2.9	
	0.31	1336	36	2.7	83	6	7.1	16
	0.63	2183	84	3.8	112	3	2.9	20
	1.3	3092	102	3.3	161	2	1.3	19
	2.5	3749	133	3.5	216	8	3.5	17
	5.0	4354	46	1.1	271	3	1.1	16
	10	4626	154	3.3	316	9	3.0	15
	20	4876	223	4.6	353	11	3.1	14
MDM2	0	27	1	2.7	34	3	9.5	
	0.31	967	25	2.5	109	4	3.3	8.9
	0.63	1778	35	2.0	172	4	2.2	10
	1.3	3452	93	2.7	293	11	3.8	12
	2.5	6248	131	2.1	517	18	3.4	12
	5.0	11387	581	5.1	904	20	2.3	13
	10	18580	737	4.0	1520	5	0.3	12
	20	28482	625	2.2	2449	79	3.2	12

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 µg/well or less without compromising speed or performance
- **Large dynamic range**: Linear range of up to five logs enables the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions
- **Minimal background:** The stimulation mechanism (electricity) is decoupled from the signal (light)
- **Simple protocols:** Only labels near the electrode surface are detected, enabling no-wash assays
- **Flexibility:** Labels are stable, non-radioactive, and conveniently conjugated to biological molecules
- **High sensitivity and precision:** Multiple excitation cycles of each label enhance light levels and improve sensitivity

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References:

1. Uhrinova S, Uhrin D, Powers H, Watt K, Zheleva D, Fischer P, McInnes C, Barlow PN. Structure of free MDM2 N-terminal domain reveals conformational adjustments that accompany p53-binding. J Mol Biol. 2005 Jul 15;350(3):587-98.
2. Inuzuka H, Fukushima H, Shaik S, Wei W. Novel Insights into the Molecular Mechanisms Governing Mdm2 Ubiquitination and Destruction. Oncotarget. 2010 Nov;1(7):685-90.
3. Stommel JM, Wahl GM. Accelerated MDM2 autodegradation induced by DNA-damage kinases is required for p53 activation. Embo J. 2004 Apr 7;23(7):1547-56.
4. Wade M, Wang YV, Wahl GM. The p53 orchestra: Mdm2 and Mdmx set the tone. Trends Cell Biol. 2010 May;20(5):299–309.
5. Marine JC, Francoz S, Maetens M, Wahl G, Toledo F, Lozano G. Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. Cell Death Differ. 2006 Jun;13(6):927–34.