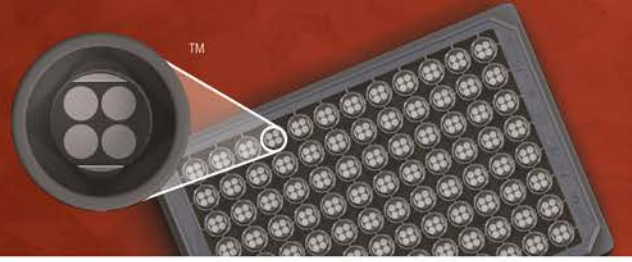
MSD® Ubiquitinated/Total p53 Assay Whole Cell Lysate Kit

For quantitative determination in human, mouse, and rat 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 p53 Whole Cell Lysate Kit	
Kit size	
1 plate	K15169D-1
5 plates	K15169D-2
20 plates	K15169D-3

Ubiquitinated p53 Whole Cell Lysate Set	
200 µg	C11FK-1

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

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

www.mesoscale.com®

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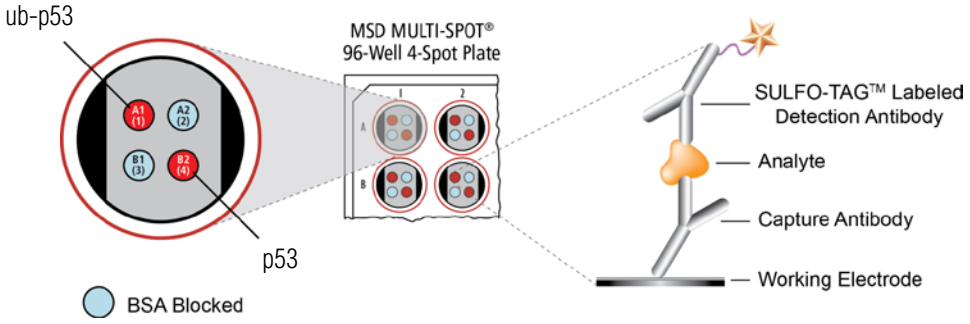

p53 (protein 53) is a transcription factor and tumor suppressor protein with an apparent molecular weight of 53 kDa, which plays a critical role in cell cycle regulation, progression, and apoptosis.[1] MDM2 is a potent negative regulator of p53 through its binding and subsequent polyubiquitination of p53, resulting in proteasome dependent degradation.[2] This negative regulation can be relieved both through phosphorylation of p53, resulting in destabilization of the MDM2-p53 interaction,[3] and through phosphorylation and ubiquitination of MDM2.[1] p53 is the most commonly mutated gene in cancer, and a functional copy of p53 is required to maintain a non-tumorigenic phenotype.[4] When cell repair is possible, p53 activates genes which pause the cell cycle allowing time for DNA repair, but when damage is extensive p53 activates the BCL-2 family of proteins leading to apoptosis.[5] p53's role as a transcription factor and the negative regulation of the protein by MDM2 mediated polyubiquitination has been extensively researched due to its crucial role in cancer prevention and cell cycle control.

The MSD Ubiquitinated/Total p53 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 p53 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 p53 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 p53 antibody on spatially distinct electrodes within a well. Ubiquitinated and total p53 were detected with anti-total p53 antibody conjugated with MSD SULFO-TAG™ reagent. Ub-p53 electrode allows detection of directly ubiquitinated p53, and also potentially p53 complexed with other ubiquitinated proteins.

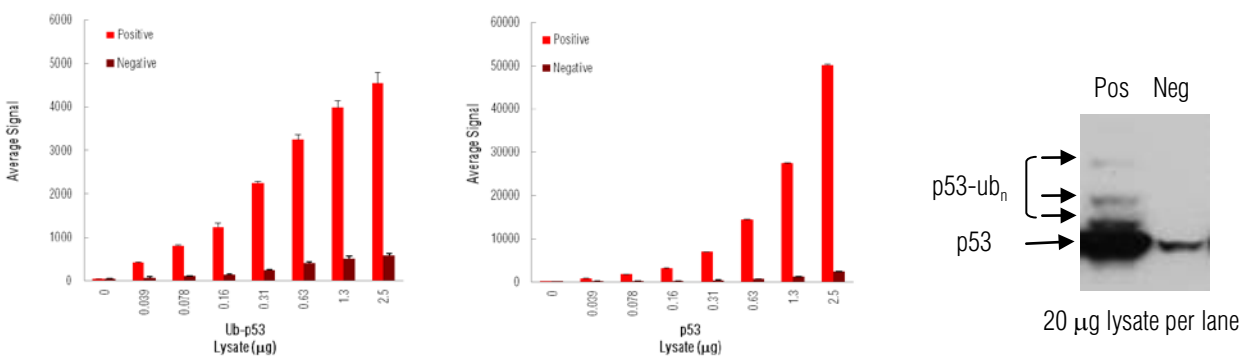

Fig. 1: Sample data generated with MULTI-SPOT Ubiquitinated/Total p53 Assay. Increased signal was observed with the titration of p53 positive cell lysate. Signal for the negative lysate remains low throughout the titration. The Ubiquitinated/Total p53 Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative HCT116 cell lysates using the MULTI-SPOT Ubiquitinated/Total p53 Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
ub-p53	0	41	10	25.6	47	11	23.2	
	0.039	411	15	3.7	78	19	24.0	5.3
	0.078	808	14	1.7	115	3	2.2	7.0
	0.16	1235	86	7.0	152	2	1.3	8.1
	0.31	2260	33	1.5	244	12	4.7	9.3
	0.63	3252	108	3.3	404	20	4.9	8.0
	1.3	3994	154	3.9	516	62	12.1	7.7
	2.5	4550	238	5.2	587	39	6.7	7.8
p53	0	38	16	41.4	37	14	38.2	
	0.039	897	21	2.3	90	1	1.1	10
	0.078	1861	35	1.9	132	8	5.7	14
	0.16	3100	315	10.2	200	27	13.5	15
	0.31	6984	469	6.7	362	21	5.8	19
	0.63	14481	638	4.4	713	12	1.7	20
	1.3	27489	1782	6.5	1263	155	12.3	22
	2.5	50122	1751	3.5	2383	226	9.5	21

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

For a complete list of products, please visit our website at www.mesoscale.com

References:

1. Hiroyuki Inuzuka, Hidefumi Fukushima, Shavali Shaik, and Wenyi Wei, Novel insights into the molecular mechanisms governing Mdm2 ubiquitination and destruction. Oncotarget 2010 Nov;1(17):685-90..
2. Midgley CA, Lane DP. p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. Oncogene. 1997 Sep 4;15(10):1179-89.
3. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, Liu D, Elledge SJ, Mak TW. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000 Mar 10;287(5459):1824-7.
4. Kenzelmann Broz D, Attardi LD. In vivo analysis of p53 tumor suppressor function using genetically engineered mouse models. Carcinogenesis. 2010 Aug;31(8):1311-8.
5. Brady CA, Attardi LD. p53 at a glance. J Cell Sci. 2010 August 1; 123(Pt 15): 2527–32.