MSD® Ubiquitinated 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 p53 Whole Cell Lysate Kit				
Kit size				
1 plate	K151FKD-1			
5 plates	K151FKD-2			
20 plates	K151FKD-3			

Ubiquitinated p53				
Whole Cell Lysate Set				
200 μ g	C11FK-1			

Ordering information

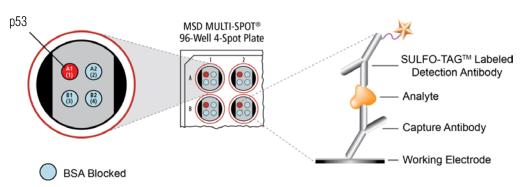
MSD Customer Service Phone: 1-301-947-2085 Fax: 1-301-990-2776 Email: CustomerService@ mesoscale.com

Company Address

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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. MDM2 is a potent negative regulator of p53 through its binding and subsequent polyubiquitination of p53, resulting in proteasome dependent degradation. This negative regulation can be relieved both through phosphorylation of p53, resulting in destabilization of the MDM2-p53 interaction, and through phosphorylation and ubiquitination of MDM2. p53 is the most commonly mutated gene in cancer, and a functional copy of p53 is required to maintain a non-tumorigenic phenotype. 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. 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 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 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 for comparison. Growing HCT116 cells (negative) were treated with doxorubicin (1 μ M; 21 hours) and epoxomicin (1 μ M; 6 hours) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-total p53 antibody on one of the four spatially distinct electrodes per well. Ubiquitinated p53 was detected with antibody against ubiquitinated proteins conjugated with MSD SULFO-TAGTM.

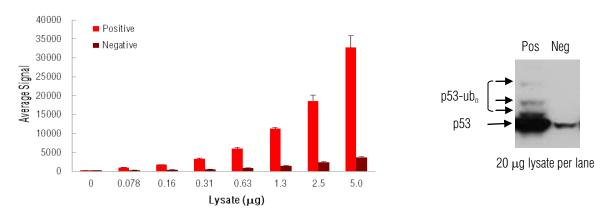


Fig. 1: Sample data generated with MULTI-ARRAY[®] Ubiquitinated p53 Assay. Increased signal is observed with the titration of ubiquitinated p53 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Ubiquitinated p53 Assay provides a quantitative measure of the data obtained with the traditional Western blot.





MSD Phosphoprotein Assays

Lysate Titration

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

Lysate	Positive			Negative			D/N
(μg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	P/N
0	245	8	3.5	243	14	5.9	1.0
0.078	1018	10	1.0	321	11	3.4	3.2
0.16	1758	50	2.8	420	11	2.7	4.2
0.31	3327	138	4.1	554	53	9.5	6.0
0.63	6006	312	5.2	858	51	5.9	7.0
1.3	11281	277	2.5	1482	57	3.8	7.6
2.5	18596	1,522	8.2	2362	173	7.3	7.9
5.0	32744	3,099	9.5	3714	141	3.8	8.8

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 μ g/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:

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- 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.
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