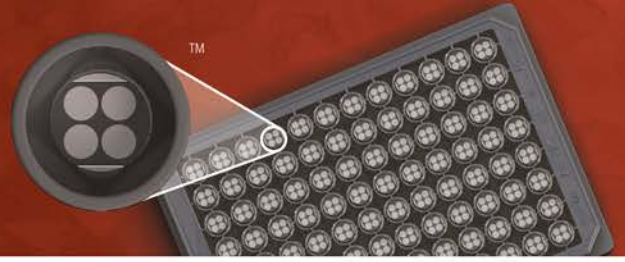
MSD® Phospho(Ser15)/Total p53 Assay Whole Cell Lysate Kit

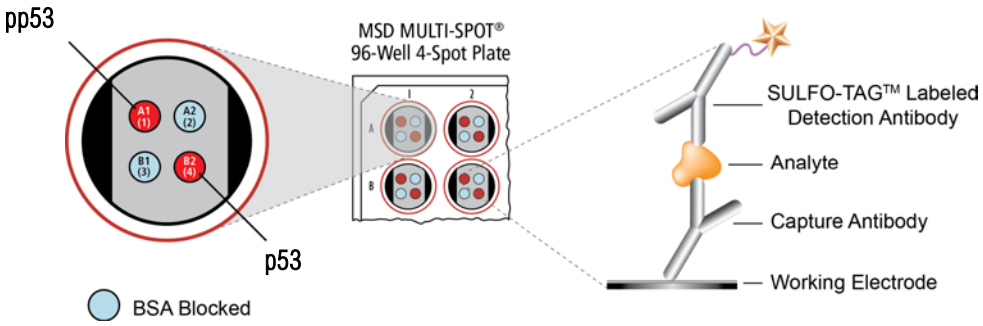
For quantitative determination in human whole cell lysate samples



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

Catalog Numbers

Phospho(Ser15)/Total p53 Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K15113D-1
5 plates	K15113D-2
20 plates	K15113D-3



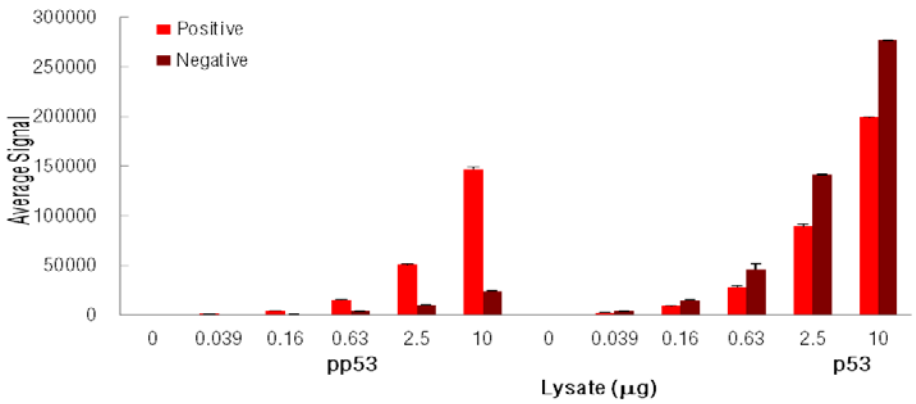
p53 (protein 53) is a transcription factor and tumor suppressor protein with an apparent molecular weight of 53 kDa that plays a critical role in cell cycle regulation, progression, and apoptosis.[1] MDM2 (murine double minute 2) is a potent negative regulator of p53 through its binding and subsequent 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]

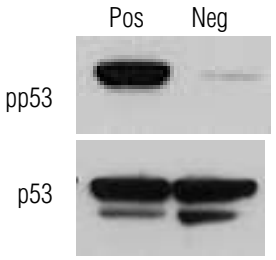
p53 is the most commonly mutated gene in cancer, and a functional copy of p53 is required to maintain a non-tumorigenic phenotype.[4] When cell repair is possible, p53 activates genes which pause the cell cycle allowing time for DNA repair, but when damage is extensive, p53 activates the BCL-2 family 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 Phospho(Ser15)/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 Phospho(Ser15)/Total p53 Assay are illustrated below. The signal and ratio values provided are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-p53 (Ser15) and total p53 antibodies and are shown for comparison.
Growing HT29 cells (negative) were harvested 1 hour after UV irradiation (40 mJ/cm^2) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-p53 (Ser15) antibody and anti-total p53 antibody on spatially distinct electrodes within a well. Phosphorylated and total p53 were detected with anti-total p53 antibody conjugated with MSD SULFO-TAG™.





20 µg lysate per lane

Fig. 1: Sample data generated with the MULTI-SPOT Phospho(Ser15)/Total p53 Assay. Increased signal for phosphorylated p53 was observed with only pp53 positive cell lysate. Total p53 signal increased throughout the titration of both pp53 positive and negative cell lysates. The Phospho(Ser15)/Total p53 Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Ordering information

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Spot the Difference™

Lysate Titration

Data for pp53 positive and negative HT29 cell lysates using the MULTI-SPOT Phospho(Ser15)/Total p53 Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pp53	0	30	5	16.8	28	9	33.4	
	0.039	1334	23	1.7	382	2	0.6	3.5
	0.16	4583	436	9.5	1219	25	2.0	3.8
	0.63	15193	319	2.1	4102	290	7.1	3.7
	2.5	51045	522	1.0	10424	85	0.8	4.9
	10	146814	3160	2.2	23871	822	3.4	6.2
p53	0	28	8	28.3	29	1	4.9	
	0.039	2634	206	7.8	4331	164	3.8	0.6
	0.16	9054	1136	12.6	14867	629	4.2	0.6
	0.63	28498	698	2.4	46364	5786	12.5	0.6
	2.5	89099	2449	2.7	141111	187	0.1	0.6
	10	199210	722	0.4	277193	28	0.0	0.7

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

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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.
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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.