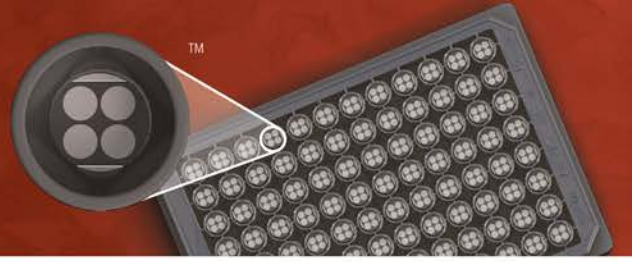
MSD® Apoptosis Panel: Whole Cell Lysate Kit



For quantitative determination of cleaved PARP (Asp214), cleaved Caspase-3 (Asp175), phosphorylated p53 (Ser15), and total p53 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

Apoptosis Panel Whole Cell Lysate Kit	
Kit size	
1 plate	K15102D-1
5 plates	K15102D-2
20 plates	K15102D-3

Ordering information

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

Company Address

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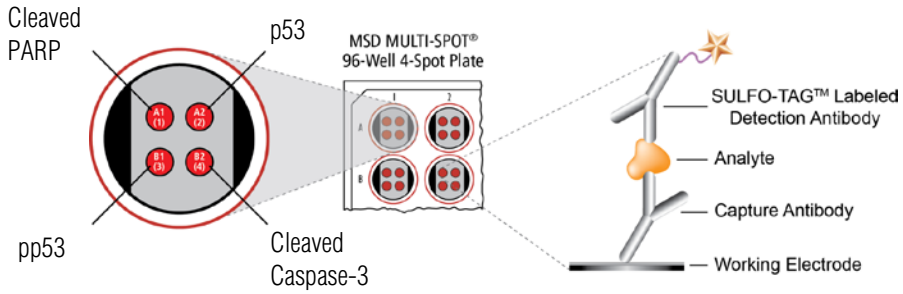

Apoptosis is the controlled or regulated destruction of a cell. The central players in apoptosis are the caspase family of cysteinyl aspartate-specific proteases, which can be sub-divided into two groups, initiator and effector caspases. Caspases 2, 8, 9, and 10 are initiator caspases and contain N-terminal adaptor domains allowing for auto-cleavage and activation of downstream effector capases, caspases 3, 6, and 7.[1] One of the substrates of caspase-3 is poly (ADP-ribose) polymerase (PARP). PARP normally promotes cell survival by repairing DNA damage, but when cellular damage is beyond repair, the apoptotic pathway mediated by the caspases inactivates PARP and ensures that severely damaged cells are destroyed.[2] p53 is the most commonly mutated gene in cancer.[3] p53 is negatively regulated by MDM2, and phosphorylation of p53 at Ser15 removes this regulation. When cell repair is possible, p53 activates genes which pause the cell cycle allowing time for DNA repair, but in cases of extensive damage, p53 activates the BCL-2 family of proteins leading to apoptosis.[4]

The MSD Apoptosis Panel Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data with Jurkat Cells Treated with Etoposide: Control Lysates for Cleaved Caspase-3 and Cleaved PARP

Representative results for the Apoptosis Panel are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Logarithmically growing Jurkat cells (negative) were treated with etoposide (25 μM; 18 hours) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-cleaved PARP (Asp214), anti-cleaved Caspase-3 (Asp175), anti total p53, and anti-phospho-p53 (Ser15) antibodies on the four spatially distinct electrodes per well. Cleaved PARP, cleaved Caspase 3, total p53, and phospho-p53 were detected with anti-PARP, anti-Caspase-3 and anti-total p53 antibodies conjugated with MSD SULFO-TAG™ reagent. Western blot analyses of each lysate type were performed with phospho-p53, total p53, cleaved and full-length Caspase-3, and cleaved and full-length PARP antibodies and are shown below for comparison.

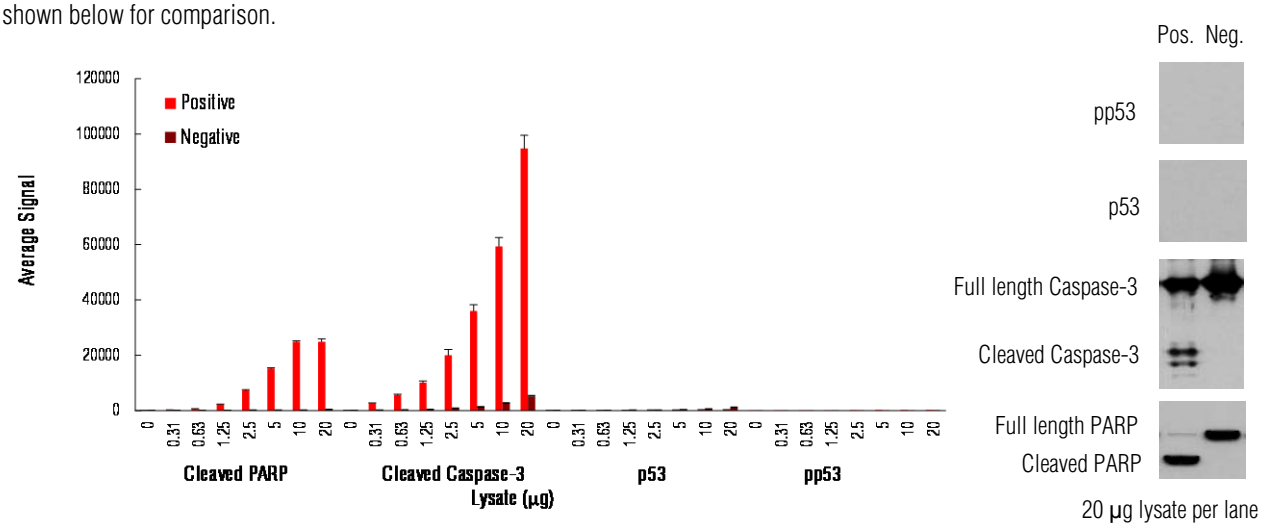

Fig. 1: Sample data generated with MULTI-SPOT Apoptosis Panel. Increased signals for cleaved PARP and cleaved Caspase-3 were observed with only Apoptosis Panel positive cell lysate. Signals for phospho- and total p53 remained low throughout the titration of positive lysate. Signals for negative lysate were also low throughout the titration for all assays. The Apoptosis Panel provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative Jurkat cell lysates using the MULTI-SPOT Apoptosis Panel are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
Cleaved PARP	0	70	15	21.1	72	7	9.1	
	0.31	216	20	9.3	76	16	21.2	2.8
	0.63	582	39	6.6	67	17	24.7	8.7
	1.3	2248	82	3.7	75	15	19.9	30
	2.5	7259	304	4.2	102	16	15.7	71
	5.0	15184	286	1.9	118	10	8.6	129
	10	24894	382	1.5	157	17	11.1	159
	20	24883	1008	4.1	325	41	12.7	76
Cleaved Caspase-3	0	45	11	23.4	47	9	19.2	
	0.31	2724	93	3.4	143	17	12.2	19
	0.63	5655	248	4.4	221	21	9.4	26
	1.3	10085	597	5.9	400	46	11.5	25
	2.5	19978	2132	10.7	764	79	10.4	26
	5.0	36055	2163	6.0	1373	24	1.7	26
	10	59357	3203	5.4	2660	86	3.2	22
	20	94707	4845	5.1	5038	308	6.1	19
p53	0	43	17	38.2	53	18	33.8	
	0.31	65	19	28.7	64	7	10.2	1.0
	0.63	77	12	15.0	83	4	4.2	0.9
	1.3	92	7	7.7	101	18	18.1	0.9
	2.5	171	29	16.9	167	9	5.6	1.0
	5.0	234	29	12.5	293	8	2.6	0.8
	10	281	49	17.6	500	52	10.4	0.6
	20	350	44	12.7	986	153	15.5	0.4
pp53	0	42	2	4.1	42	13	31.0	
	0.31	64	33	51.3	47	7	14.9	1.4
	0.63	76	21	28.2	45	10	22.2	1.7
	1.3	86	13	15.2	53	13	23.7	1.6
	2.5	155	17	11.1	71	9	12.2	2.2
	5.0	212	49	23.3	88	10	11.3	2.4
	10	213	45	21.3	93	4	4.3	2.3
	20	258	37	14.4	121	9	7.1	2.1

Typical Data with HT29 Cells Treated with UV Irradiation: Control Lysates for Phosphorylated and Total p53

Representative results for the Apoptosis Panel are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. 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-cleaved PARP (Asp214), anti-cleaved Caspase-3 (Asp175), anti total p53, and anti-phospho-p53 (Ser15) antibodies on the four spatially distinct electrodes per well. Cleaved PARP, cleaved Caspase 3, total p53, and phospho-p53 were detected with anti-PARP, anti-Caspase-3 and anti-total p53 antibodies conjugated with MSD SULFO-TAG reagent. Western blot analyses of each lysate type were performed with phospho-p53, total p53, cleaved and full-length Caspase-3, and cleaved and full-length PARP antibodies and are shown below for comparison.

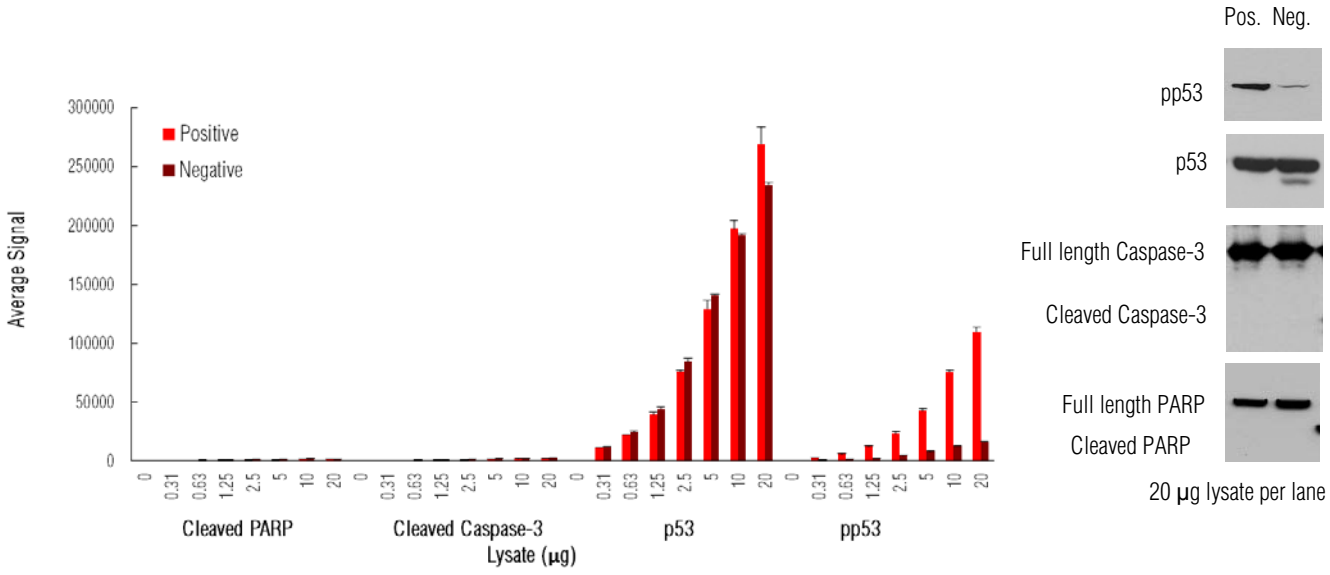

Fig. 2: Sample data generated with MULTI-SPOT Apoptosis Panel. Increased signals for phospho-p53 was observed with only Apoptosis Panel positive cell lysate, whereas total p53 signals increased throughout the titration of both positive and negative cell lysates. Cleaved PARP and cleaved Caspase-3 signals were low for both positive and negative lysates. The Apoptosis Panel provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative HT29 cell lysates using the MULTI-SPOT Apoptosis Panel are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
Cleaved PARP	0	74	11	14.3	79	15	18.7	
	0.31	338	3	0.9	473	24	5.1	0.7
	0.63	510	10	2.0	754	88	11.7	0.7
	1.3	627	13	2.0	919	62	6.7	0.7
	2.5	930	77	8.2	1384	121	8.8	0.7
	5.0	1131	65	5.8	1710	132	7.7	0.7
	10	1392	139	10.0	1964	208	10.6	0.7
	20	1346	137	10.1	1747	161	9.2	0.8

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
Cleaved Caspase-3	0	46	4	7.7	50	7	14.0	
	0.31	273	9	3.2	390	26	6.5	0.7
	0.63	457	27	5.8	646	31	4.8	0.7
	1.3	607	25	4.0	880	98	11.1	0.7
	2.5	960	81	8.4	1319	173	13.1	0.7
	5.0	1336	141	10.6	1896	189	10.0	0.7
	10	1975	52	2.6	2473	172	6.9	0.8
	20	2504	140	5.6	2990	321	10.7	0.8
p53	0	47	9	19.2	54	17	31.4	
	0.31	11357	117	1.0	12347	236	1.9	0.9
	0.63	21965	602	2.7	24519	1247	5.1	0.9
	1.3	39540	1953	4.9	44097	1470	3.3	0.9
	2.5	75930	1404	1.8	84456	2425	2.9	0.9
	5.0	128996	7564	5.9	140694	713	0.5	0.9
	10	197309	7096	3.6	192061	507	0.3	1.0
	20	269034	14612	5.4	234171	1861	0.8	1.1
pp53	0	36	7	19.4	47	3	6.9	
	0.31	3037	324	10.7	836	23	2.7	3.6
	0.63	6221	328	5.3	1568	132	8.4	4.0
	1.3	12464	594	4.8	2402	60	2.5	5.2
	2.5	23293	1849	7.9	4616	509	11.0	5.0
	5.0	42591	1774	4.2	8316	168	2.0	5.1
	10	75668	1267	1.7	12872	186	1.4	5.9
	20	110010	3671	3.3	16155	543	3.4	6.8

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:

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2. Decker P, Muller S. Modulating poly (ADP-ribose) polymerase activity: potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. Curr Pharm Biotechnol. 2002 Sep;3(3):275-83.
3. 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. Epub 2010 Jan 22.
4. Brady CA, Attardi LD. p53 at a glance. J Cell Sci. 2010 Aug 1;123(Pt 15):2527-32.