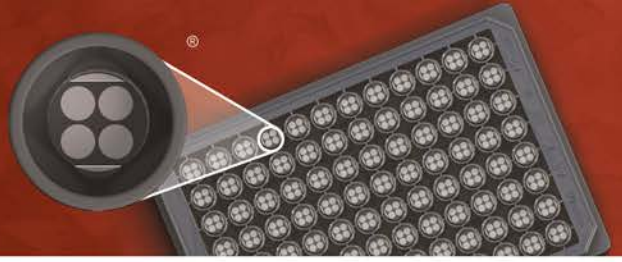
MSD® Cleaved PARP (Asp214) Assay Whole Cell Lysate Kit

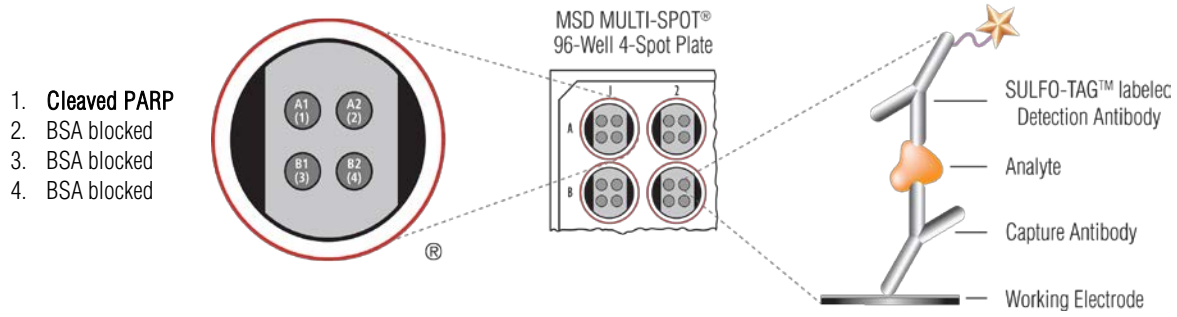
For quantitative determination in human whole cell lysate samples



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

Catalog Numbers

Cleaved PARP (Asp214) Assay Whole Cell Lysate Kit	
Kit size	
1 plate	K150DED-1
5 plates	K150DED-2
20 plates	K150DED-3

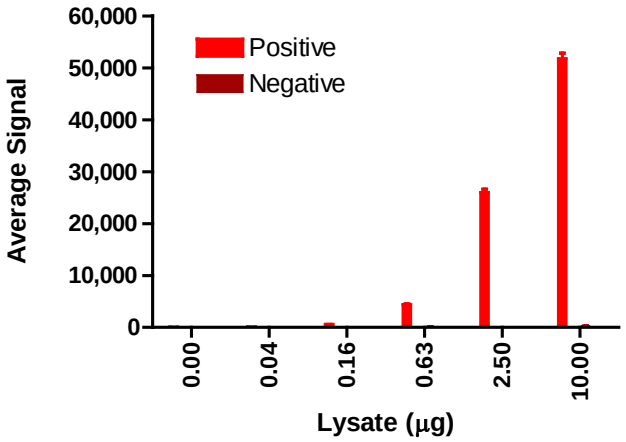


Poly (ADP-ribose) polymerase (PARP) is a highly abundant nuclear enzyme involved in DNA repair that synthesizes poly ADP ribose from NAD+ in response to damaged DNA. When cells are exposed to a critical level of damaging agents, cellular apoptosis is induced through an intracellular signaling cascade. Activation of the caspase family of apoptosis-specific proteases results in the cleavage of PARP by caspase-3. Cleavage of PARP produces the N-terminal 24 kDa DNA binding domain fragment, and the C-terminal 89 kDa catalytic domain fragment, rendering it inactive. The 24 kDa fragment has been shown to retain its DNA binding affinity for strand breaks, thereby inhibiting further DNA repair, ADP-ribose polymer formation, and transcription. Caspase-7 is also involved in PARP cleavage during apoptosis. High levels of PARP activity have been implicated in a caspase-independent cell death pathway through the depletion of cellular energy stores (NAD+) and the downstream effector AIF (Apoptosis-Inducing Factor).

The MSD Cleaved PARP (Asp214) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Cleaved PARP (Asp214) Assay are illustrated below. The signal and ratio values are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with cleavage-specific and total PARP antibodies and are shown for comparison.
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) antibody on one of the four spatially distinct electrodes per well. Cleaved PARP was detected with anti-total PARP antibody conjugated with MSD SULFO-TAG™ reagent.



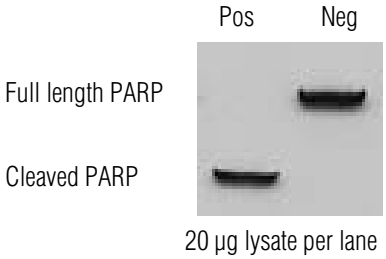


Fig. 1: Sample data generated with the MULTI-ARRAY® Cleaved PARP (Asp214) Assay. Increased signal is observed with the titration of cleaved PARP positive cell lysate. Signal for negative lysate remains low throughout the titration. The Cleaved PARP (Asp214) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Ordering information

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Lysate Titration

Data for cleaved PARP positive and negative Jurkat cell lysates using the MULTI-ARRAY Cleaved PARP (Asp214) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	75	6	7.4	66	4	6.6	1.1
0.04	114	5	4.6	67	6	9.1	1.7
0.16	566	30	5.4	81	10	11.9	7.0
0.63	4,384	217	5.0	102	12	11.7	43
2.5	25,959	1,140	4.4	116	13	10.8	223
10	51,735	1,920	3.7	257	15	6.0	202

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 using MSD's platform for the measurement of phosphoproteins

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2. Prevost GP, Lonchampt MO, Holbeck S, Attoub S, Zaharevitz D, Alley M, Wright J, Brezak MC, Coulomb H, Savola A, Huchet M, Chaumeron S, Nguyen QD, Forgez P, Bruyneel E, Bracke M, Ferrandis E, Roubert P, Demarquay D, Gespach C, Kasprzyk PG. Anticancer Activity of BIM-46174, a New Inhibitor of the Heterotrimeric Ga/Gßg Protein Complex. Cancer Res. 2006 Sep 15;66(18):9227-34.
3. Gowan SM, Hardcastle A, Hallsworth AE, Valenti MR, Hunter LJ, de Haven Brandon AK, Garrett MD, Raynaud F, Workman P, Aherne W, Eccles SA. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol. 2007 Jun;5(3):391-401.