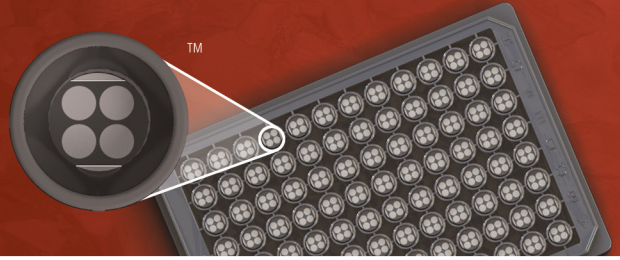
MSD® Cleaved Caspase-3 (Asp175) Assay Whole Cell Lysate Kit

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 Caspase-3 Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151CFD-1
5 plates	K151CFD-2
20 plates	K151CFD-3

Cleaved Caspase-3 Whole Cell Lysate Set	
200 µg	C11CF-1

Ordering information

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

Company Address

MESO SCALE DISCOVERY®
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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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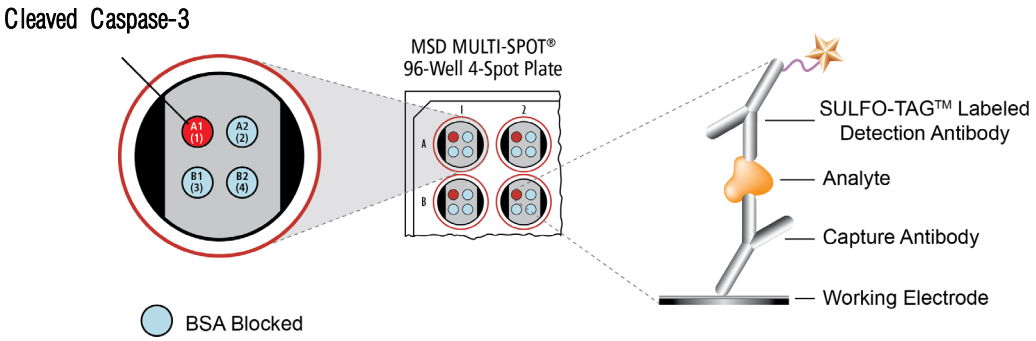
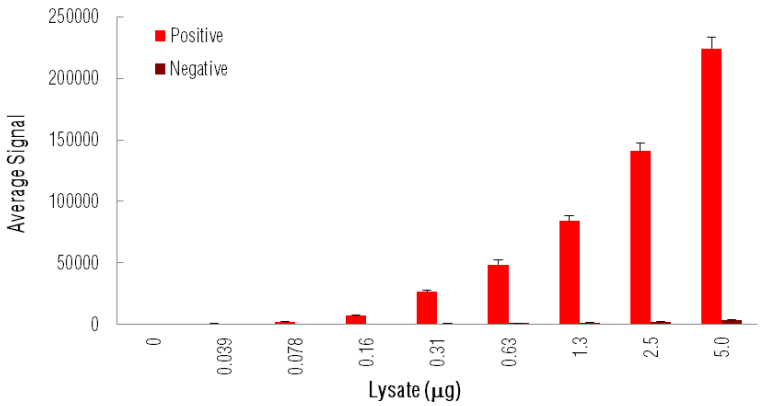


Caspase-3 (Apopain, CPP32) is part of a family of cysteinyl aspartate-specific proteases (caspases) that cleave target proteins after aspartate residues. Many caspases are involved in apoptosis signaling within the cell and are categorized as initiator or effector caspases. Caspases become activated through dimerization and proteolysis of procaspases, forming an active enzyme which contains two large and two small subunits. Caspase-3 is an effector caspase that is activated through cleavage by caspase-9, an initiator caspase activated through cytochrome c release from the mitochondria, ATP, and an interaction with Apaf-1. Active caspase-3 has been shown to cleave many proteins including Bcl-2, PARP, p21, Akt, DNA-PK, 14-3-3 proteins, and eukaryotic translation initiation factors 4G and 2α. Cleavage of caspase-3 substrates facilitates apoptosis through suppression of cell survival pathways or the amplification of intracellular apoptotic signals.

The MSD Cleaved Caspase-3 Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Cleaved Caspase-3 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 cleavage-specific and total caspase-3 antibodies and are shown below for comparison.
Logarithmically growing Jurkat cells (negative) were treated with staurosporine (1 µM; 4 hours) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-cleaved Caspase-3 antibody on one of the four spatially distinct electrodes per well. Cleaved Caspase-3 was detected with an anti-total Caspase-3 antibody conjugated with MSD SULFO-TAG™ reagent.



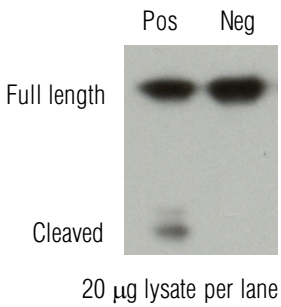


Fig. 1: Sample data generated with the MULTI-ARRAY Cleaved Caspase-3 Assay. Increased signal is observed with the titration of cleaved Caspase-3 positive cell lysate. The Cleaved Caspase-3 Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for cleaved Caspase-3 positive and negative Jurkat cell lysates using the MULTI-ARRAY Cleaved Caspase-3 Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	120	10	8.3	134	15	11.2	
0.039	543	60	11.0	133	12	9.0	4.1
0.078	1933	146	7.6	146	8	5.5	13
0.16	7013	536	7.6	223	10	4.5	31
0.31	26519	972	3.7	548	19	3.5	48
0.63	48691	3491	7.2	887	50	5.6	55
1.3	83952	4669	5.6	1417	47	3.3	59
2.5	140769	6895	4.9	2141	50	2.3	66
5.0	224303	9146	4.1	3442	132	3.8	65

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 using MSD's technology for the measurement of phosphoproteins

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3. Sasiela CA, Stewart DH, Kitagaki J, Safiran YJ, Yang Y, Weissman AM, Oberoi P, Davydov IV, Goncharova E, Beutler JA, McMahon JB, O'Keefe BR. Identification of inhibitors for MDM2 ubiquitin ligase activity from natural product extracts by a novel high-throughput electrochemiluminescent screen. J Biomol Screen. 2008 Mar;13(3):229-37. Epub 2008 Feb 12.
4. 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.