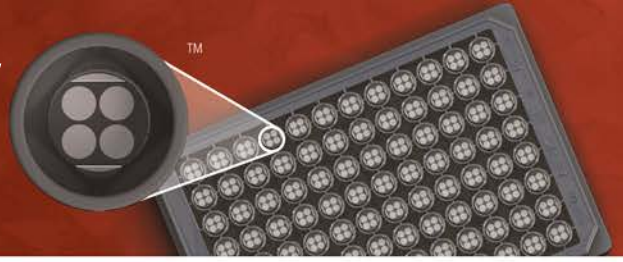
MSD® Cleaved (Asp175)/Total Caspase-3 Assay Whole Cell Lysate Kit

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/Total Caspase-3 Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K15140D-1
5 plates	K15140D-2
20 plates	K15140D-3

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

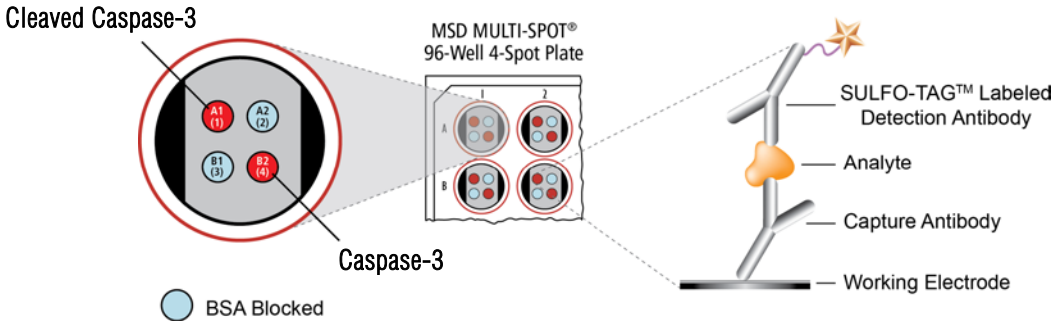
Ordering information

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

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA

www.mesoscale.com®



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/Total 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/Total 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 and anti-total Caspase-3 antibodies on two of the four spatially distinct electrodes per well. Cleaved and total Caspase-3 were detected with an anti-Caspase-3 antibody conjugated with MSD SULFO-TAG™ reagent.

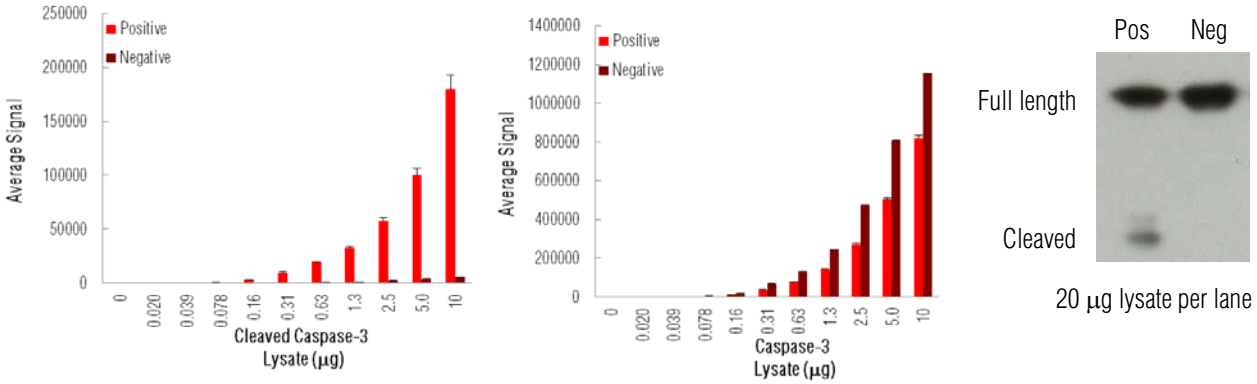


Fig. 1: Sample data generated with the MULTI-SPOT Cleaved/Total Caspase-3 Assay. Increased signal for cleaved Caspase-3 was observed with only cleaved Caspase-3 positive cell lysate. Total Caspase-3 signal increased throughout the titration of both positive and negative cell lysates. The Cleaved/Total 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-SPOT Cleaved/Total Caspase-3 Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
Cleaved Caspase-3	0	94	6	6.0	111	8	7.4	
	0.020	139	11	7.7	100	8	7.5	1.4
	0.039	270	20	7.5	107	4	3.6	2.5
	0.078	783	64	8.2	122	12	9.5	6.4
	0.16	2751	233	8.5	204	7	3.3	14
	0.31	9711	580	6.0	420	14	3.4	23
	0.63	18858	791	4.2	674	7	1.0	28
	1.3	32443	1423	4.4	1086	34	3.1	30
	2.5	57339	3129	5.5	2042	166	8.2	28
	5.0	99895	6760	6.8	3673	290	7.9	27
	10	179776	12890	7.2	5470	187	3.4	33
Caspase-3	0	153	10	6.6	183	9	5.0	
	0.020	282	9	3.1	374	16	4.2	0.8
	0.039	687	33	4.8	1147	21	1.8	0.6
	0.078	2321	160	6.9	4244	67	1.6	0.5
	0.16	9426	564	6.0	17177	299	1.7	0.5
	0.31	36914	2196	6.0	65721	1425	2.2	0.6
	0.63	76386	4192	5.5	128767	2908	2.3	0.6
	1.3	141237	5637	4.0	244098	5835	2.4	0.6
	2.5	272437	5591	2.1	472086	8291	1.8	0.6
	5.0	504516	14863	2.9	808693	29575	3.7	0.6
	10	820994	41101	5.0	1153263	29937	2.6	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

For a complete list of products, please visit our website at www.mesoscale.com