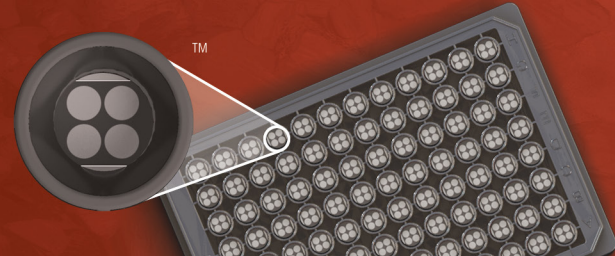


MSD® Phospho-NFκB (Ser468) Assay Whole Cell Lysate Kit

For quantitative determination in human, mouse, and rat whole cell lysate samples



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

Catalog Numbers

Phospho-NFκB (Ser468)
Assay: Whole Cell Lysate Kit

Kit size

1 plate	K151EDD-1
5 plates	K151EDD-2
20 plates	K151EDD-3

Phospho-NFκB Whole Cell
Lysate Set

200 μg	C11ED-1
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Ordering information

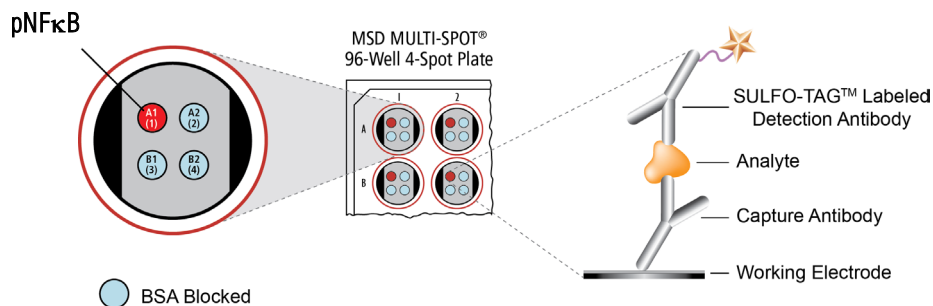
MSD Customer Service
Phone: 1-301-947-2085
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Email: CustomerService@mesoscale.com

Company Address

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NFκB/p65 (nuclear factor kappa-light-chain-enhancer of activated B cells) refers to a family of five transcription factors which function downstream of receptors such as TNFR and the toll-like receptor superfamily. These transcription factors are involved in inflammation, immune response and development, proliferation, and cell survival.¹ Inactive NFκB is normally found in the cytoplasm, bound by IκB (inhibitor of kappa B). Upon stimulation of an upstream receptor, IκB becomes phosphorylated and ubiquitinated and releases the NFκB which can translocate to the nucleus and function as a transcription factor.² NFκB is activated by diverse stimuli such as reactive oxygen species, IL-1β, TNF, bacterial lipopolysaccharide, and ionizing radiation to name a few.¹

NFκB stimulation is generally proliferative, proinflammatory, and anti-apoptotic and its upregulation in human cancers promotes tumor proliferation, metastasis, and chemoresistance.³ Unregulated NFκB activity also contributes to a number of inflammatory conditions, such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis.³ NFκB dysregulation has been seen in cancers of lymphoid origin, colitis associated cancers, hepatocellular carcinomas, and prostate cancer.⁴ Because of the relationship between NFκB and many diseased states, there has been much research into regulation of NFκB and the upstream members of this signal transduction cascade.⁵

The MSD Phospho-NFκB (Ser468) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-NFκB (Ser468) 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 phospho-NFκB and total NFκB antibodies and are shown below for comparison.

Growing Jurkat cells (negative) were treated with TNFα (20 ng/mL; 15 minutes) and Calyculin A (50 nM; 15 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-NFκB antibody on one of the four spatially distinct electrodes per well. Phosphorylated NFκB was detected with anti-total NFκB antibody conjugated with MSD SULFO-TAG™ reagent.

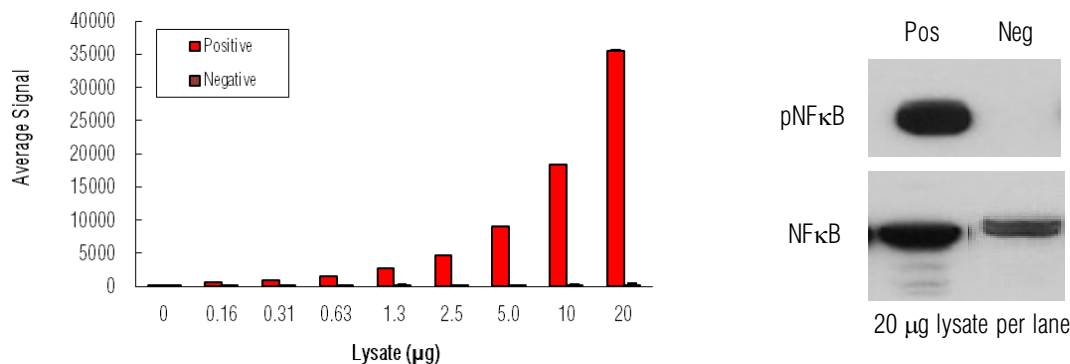


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-NFκB (Ser468) Assay. Increased signal is observed with the titration of pNFκB positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-NFκB (Ser468) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

MSD Phosphoprotein Assays

Lysate Titration

Data for pNF κ B positive and negative Jurkat cell lysates using the MULTI-ARRAY Phospho-NF κ B (Ser468) Assay are presented below.

Lysate (μ g)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	140	7	5	138	6	4	
0.16	575	15	3	178	5	3	3.2
0.31	884	18	2	157	20	13	5.6
0.63	1553	56	4	165	10	6	9.4
1.3	2676	123	5	170	11	6	16
2.5	4714	26	1	194	9	4	24
5.0	9088	12	0	195	6	3	47
10	18293	158	1	213	9	4	86
20	35496	193	1	216	3	1	164

MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25 μ g/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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