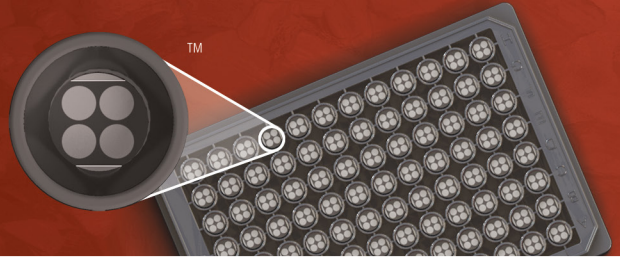
MSD® Phospho-NFκB (Ser536) Assay Whole Cell Lysate Kit

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 (Ser536) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K151ECD-1
5 plates	K151ECD-2
20 plates	K151ECD-3

Phospho-NFκB Whole Cell Lysate Set	
200 µg	C11ED-1

Ordering information

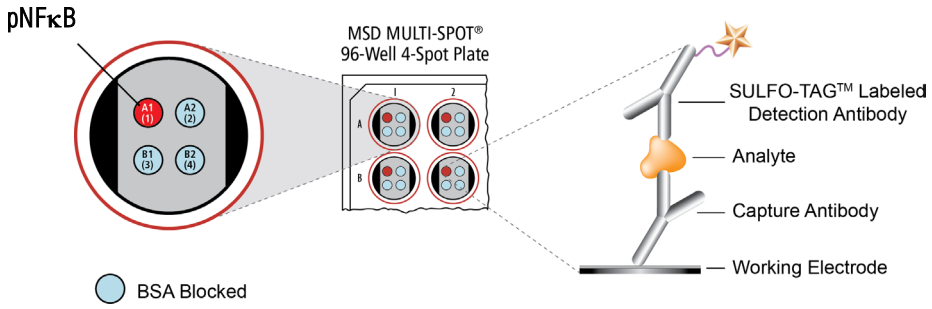
MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

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

www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures.



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 (Ser536) 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 (Ser536) 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.

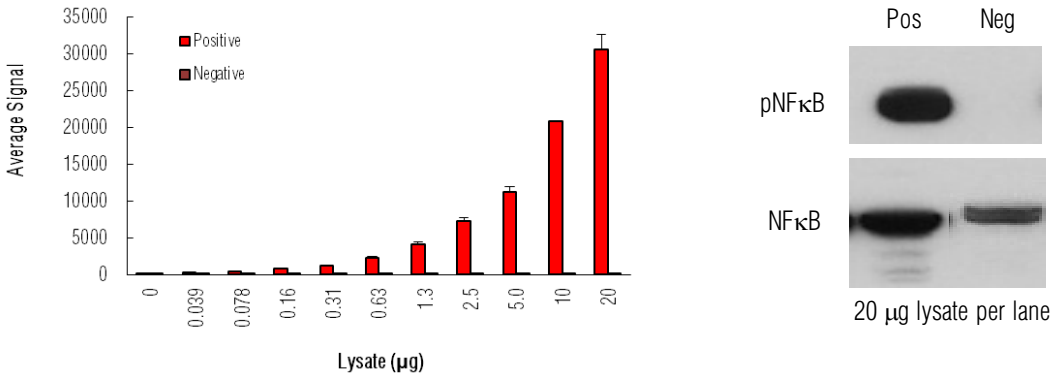


Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-NFκB (Ser536) Assay. Increased signal is observed with the titration of pNFκB positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-NFκB (Ser536) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pNFκB positive and negative Jurkat cell lysates using the MULTI-ARRAY Phospho-NFκB (Ser536) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	20	11	53	32	8	24	
0.039	239	12	5	31	6	18	7.7
0.078	415	34	8	51	10	19	8.2
0.16	745	33	4	54	7	12	14
0.31	1249	32	3	35	12	35	35
0.63	2251	171	8	44	9	20	51
1.3	4149	317	8	61	2	3	68
2.5	7235	499	7	69	8	12	104
5.0	11214	690	6	77	12	15	145
10	20844	7	0	87	11	12	239
20	30595	2006	7	100	15	15	305

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

1. O'Dea E, Hoffmann A. The Regulatory Logic of the NFkB Signaling System. Cold Spring Harb Perspect Biol. 2010 January; 2(1): a000216.
2. Hoffmann A, Leung TH, Baltimore D. Genetic analysis of NF-kappaB/Rel transcription factors defines functional specificities. Embo J 2003;22:5530–5539. [PubMed: 14532125]
3. O'Dea E, Hoffmann A. NF-κB signaling. Wiley Interdiscip Rev Syst Biol Med. 2009 July 1; 1(1): 107.
4. Karin M. NF-kappaB as a critical link between inflammation and cancer. Cold Spring Harb Perspect Biol. 2009 Nov;1(5):a000141.
5. Escárcega RO, Fuentes-Alexandro S, García-Carrasco M, Gatica A, Zamora A. The transcription factor nuclear factor-kappa B and cancer. Clin Oncol (R Coll Radiol). 2007 Mar;19(2):154-61.