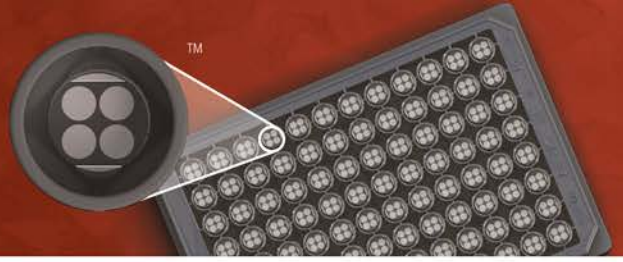
MSD® Phospho-Pleckstrin (Ser) Whole Cell Lysate Kit

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-Pleckstrin (Ser) Whole Cell Lysate Kit	
Kit size	
1 plate	K150LED-1
5 plates	K150LED-2
25 plates	K150LED-4

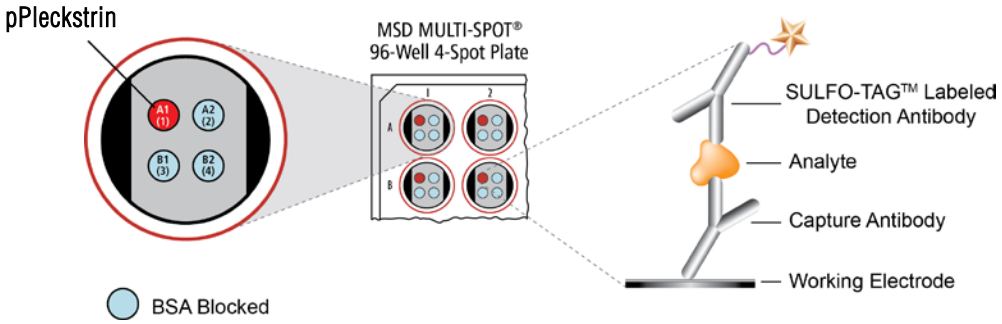
Ordering information

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®



Pleckstrin, a 40 kDa phosphoprotein found in platelets and leukocytes, is a prominent substrate of protein kinase C (PKC) in platelets, monocytes, macrophages, lymphocytes, and granulocytes. Pleckstrin accounts for 1% of the total protein in these cells and is used as an early marker of platelet activation.[1,2] The pleckstrin molecule is divided into three motifs: Pleckstrin homology (PH) domains at the NH2 and COOH termini and an intervening disheveled-Egl 10-pleckstrin (DEP) domain. A short stretch of amino acids between the NH2-terminal PH domain and the DEP domain contains three oxygenated residues (Ser113, Thr114, and Ser117) that are phosphorylated by PKC and are essential for regulating pleckstrin's ability to interact with its lipid and protein-binding partners.[1-3]
The PH domains constitute phosphoinositide-binding motifs and following phosphorylation, play a role in targeting a vast number of PH domain-containing proteins to appropriate intracellular compartments to enable further protein interactions or signaling cascades. These interactions regulate cellular homeostasis. Dysregulation can result in progressive pathogenic states such as cell transformation and tumorigenesis.[2,3] Pleckstrin is also involved in changes in cell morphology, actin activation, and PKC-mediated exocytosis of platelet granules.[1,3] Defects in pleckstrin phosphorylation have been directly linked to cardiovascular disease, increased phagocytic damage, and proinflammatory cytokine release during hyperglycemia and diabetes.[4]
The MSD Phospho-Pleckstrin (Ser) assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-Pleckstrin (Ser) assay are illustrated below. The signal and ratio values provided are examples; individual results may vary depending upon the samples tested.
Growing Jurkat cells were treated with PMA (200 nM) and Calyculin A (50 nM) for 30 minutes (positive) or with rapamycin (1 μM) for 3 hours (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-spot plates coated with anti-total pleckstrin antibody on one of the four spatially distinct electrodes in each well. Phosphorylated pleckstrin was detected with anti-phospho-pleckstrin (Ser) antibody conjugated with MSD SULFO-TAG™.

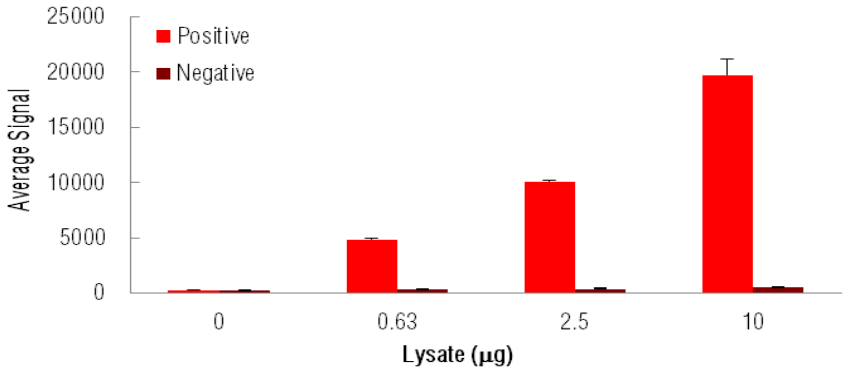


Fig. 1: Sample data generated with the Phospho-Pleckstrin (Ser) assay. Increased signal was observed with the titration of phospho-pleckstrin positive cell lysate. Signal for negative lysate remains low throughout the titration.

Lysate Titration

Data for phospho-pleckstrin positive and negative Jurkat cell lysates using the Phospho-Pleckstrin (Ser) assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	243	12	5.0	239	16	6.8	
0.63	4846	108	2.2	301	4	1.2	16
2.5	10075	132	1.3	382	6	1.5	26
10	19718	1482	7.5	521	23	4.5	38

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

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2. Guy GR, Yusoff P, Bangarusamy D, Fong CW, Wong ES. Dockers at the crossroads. Cell Signal. 2002 Jan;14(1):11-20.
3. Sloan DC, Wang P, Bao X, Haslam RJ. Translocation of pleckstrin requires its phosphorylation and newly formed ligands. Biochem Biophys Res Commun. 2002 293(1):640-646.
4. Ding Y, Kantarci A, Badwey JA, Hasturk H, Malabanan A, Van Dyke TE. Phosphorylation of pleckstrin increases proinflammatory cytokine secretion by mononuclear phagocytes in diabetes mellitus. J Immunol. 2007 Jul;179(1):647-54.