MSD® Insulin Signaling Panel (Phospho Protein) Whole Cell Lysate Kit

For quantitative determination of phosphorylated IGF-1R, IR, and IRS-1 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

Insulin Signaling Panel Whole Cell Lysate Kit	
Kit size	
1 plate	K15151C-1
5 plates	K15151C-2
20 plates	K15151C-3

Insulin Signaling Panel Whole Cell Lysate Set	
200 µg	C1151-1

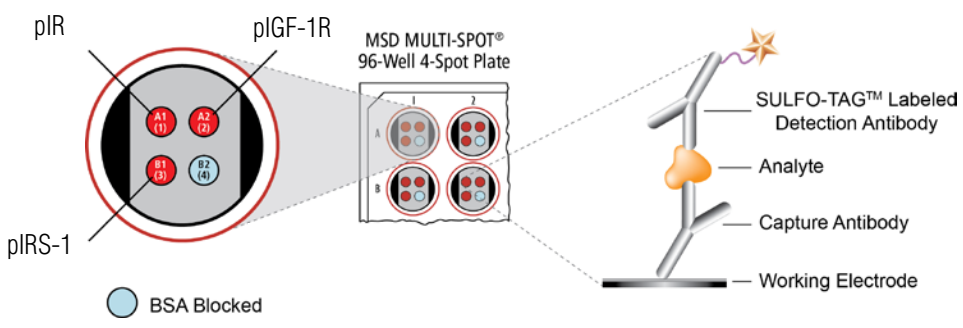## Ordering information

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IGF (Insulin like Growth Factor) signaling is mediated by the ligands IGF1 and IGF2, and the receptors **IGF-1R (Insulin like Growth Factor-1 Receptor)**, IGF-2R, and **IR (Insulin Receptor)**. Additionally, downstream signaling from IGF-1R/IR through the PI3K signaling pathway is accomplished due to the adaptor protein **IRS-1 (Insulin Receptor Substrate-1)** binding to the phosphorylated and activated IGF-1R or IR.[1] Upon ligand binding, these receptors autophosphorylate on multiple tyrosine residues within the beta subunits.[2] This results in the binding of multiple docking and adaptor proteins (such as Shc and IRS-1/2) involved in signaling through the Ras/MAP kinase and AKT/PI3 kinase signaling cascades.[3]

IR and IGF-1R signaling play a key role in normal development, growth, metabolism, and cellular homeostasis as well as being implicated in such disease processes as growth abnormalities, metabolic processes, and different types of cancer.[4] Pharmacological disruption of IGF-1R/IR signaling is a fertile area of research, but due a critical role in normal cellular metabolism and growth, the ability to identify selective therapeutics targeting abnormally signaling cells would be greatly advantageous.[5]

The MSD Insulin Signaling Panel Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Insulin Signaling Panel 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-specific antibodies for the indicated phosphorylation sites(s) on each target and are shown below for comparison.

Serum deprived MCF7 cells (negative) were treated with IGF-1 (100 nM, 15 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total IR, anti-total IGF-1R, and anti-total IRS-1 antibodies on three of the four spatially distinct electrodes per well. Phosphorylated IR, IGF-1R, and IRS-1 were detected with anti-phosphotyrosine antibody conjugated with MSD SULFO-TAG™ reagent.
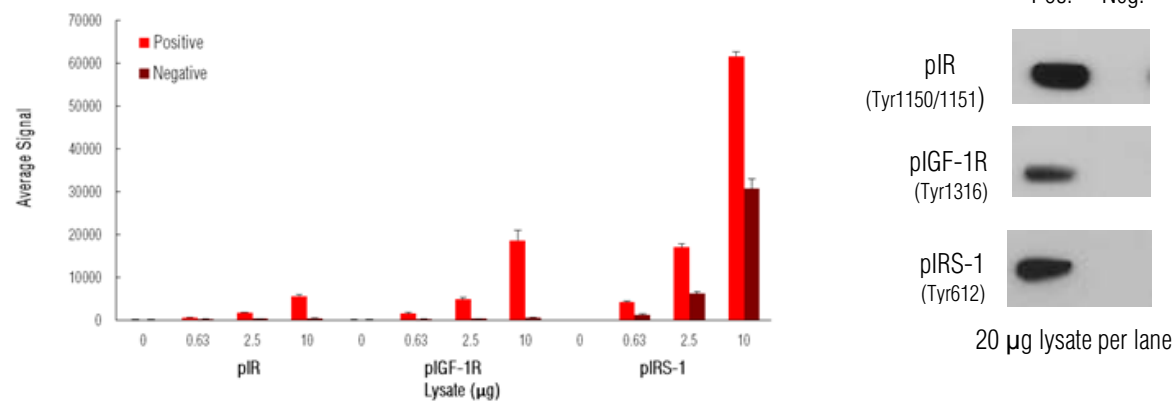

Fig. 1: Sample data generated with MULTI-SPOT Insulin Signaling Panel. Increased signals for phosphorylated forms of IR, IGF-1R, and IRS-1 were observed with Insulin Signaling Panel positive cell lysate.

Lysate Titration

Data for positive and negative MCF7 cell lysates using the MULTI-SPOT Insulin Signaling Panel are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pIR	0	177	12	6.9	174	17	9.6	
	0.63	696	55	7.9	302	22	7.2	2.3
	2.5	1804	28	1.6	381	12	3.1	4.7
	10	5645	347	6.2	511	45	8.7	11
IGF-1R	0	178	9	5.1	185	4	2.1	
	0.63	1609	308	19.1	348	28	8.2	4.6
	2.5	4933	402	8.1	443	13	2.8	11
	10	18658	2388	12.8	636	47	7.3	29
pIRS-1	0	109	2	1.4	104	9	8.6	
	0.63	4253	302	7.1	1354	157	11.6	3.1
	2.5	17069	806	4.7	6215	436	7.0	2.7
	10	61600	1099	1.8	30747	2203	7.2	2.0

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:

1. Li R, Pourpak A, Morris SW. Inhibition of the insulin-like growth factor-1 receptor (IGF1R) tyrosine kinase as a novel cancer therapy approach. J Med Chem. 2009 August 27; 52(16): 4981–5004.
2. Siddle K. Signalling by insulin and IGF receptors: supporting acts and new players. J Mol Endocrinol. 2011 Jun 17;47(1):R1-10.
3. Cohen P. The twentieth century struggle to decipher insulin signalling. Nat Rev Mol Cell Biol. 2006 Nov;7(11): 867–73.
4. Frasca F, Pandini G, Sciacca L, Pezzino V, Squatrito S, Belfiore A, and Vigneri R. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Arch Physiol Biochem. 2008 Feb;114(1):23–37.
5. Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr Rev. 2009 October;30(6):586–623.