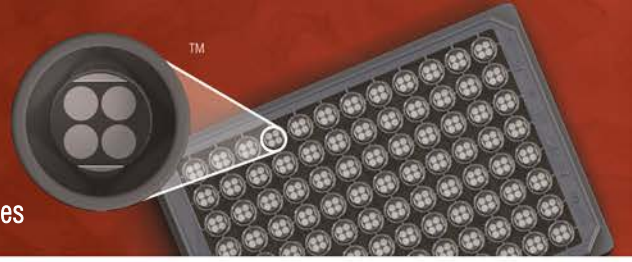
MSD® Insulin Signaling Panel (Total Protein) Whole Cell Lysate Kit

For quantitative determination of 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 (Total Protein) Whole Cell Lysate Kit	
Kit size	
1 plate	K15152C-1
5 plates	K15152C-2
20 plates	K15152C-3

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

Ordering information

MSD Customer Service
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Company Address

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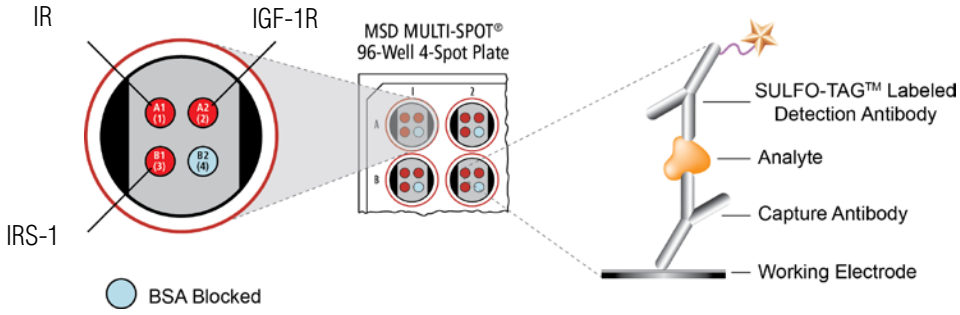

IGF (Insulin like Growth Factor) signaling is mediated by the ligands IGF1 and IGF2, and the receptors **IGF-1R (Insulin like Growth Factor-1 Receptor)**, IGF-2R, and **IR (Insulin Receptor)**. Additionally, downstream signaling from IGF-1R/IR through the PI3K signaling pathway is accomplished due to 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 (Total Protein) 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 (Total Protein) 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 total IR, total IGF-1R, and total IRS-1 antibodies 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. IR, IGF-1R, and IRS-1 were detected with anti-total IR, anti-total IGF-1R, and anti-total IRS-1 antibodies conjugated with MSD SULFO-TAG™ reagent.

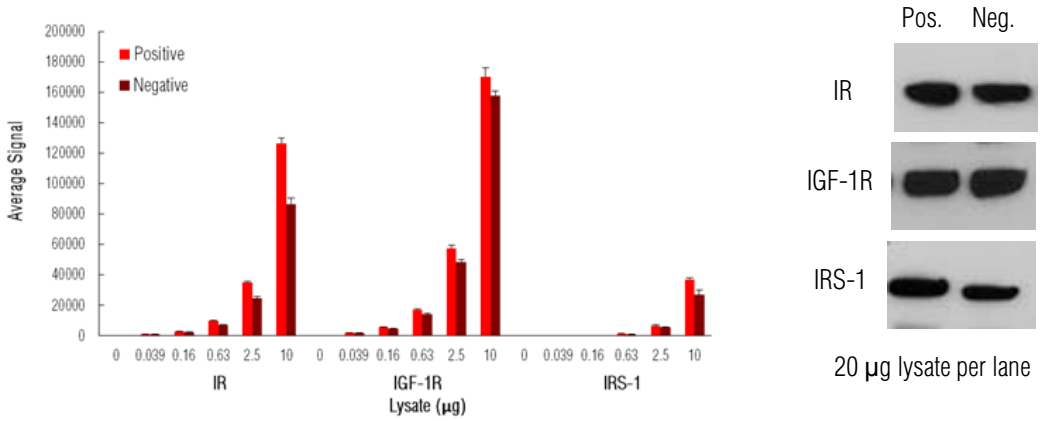

Fig. 1: Sample data generated with MULTI-SPOT Insulin Signaling Panel (Total Protein). Increased signals for IR, IGF-1R, and IRS-1 were observed with both positive and negative cell lysates. The Insulin Signaling Panel (Total Protein) provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

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

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
IR	0	266	17	6.4	209	6	2.8	
	0.039	1015	65	6.4	922	10	1.1	1.1
	0.16	2827	95	3.4	2257	24	1.1	1.3
	0.63	9716	401	4.1	7032	321	4.6	1.4
	2.5	35210	547	1.6	24499	1118	4.6	1.4
	10	126477	3621	2.9	86615	3756	4.3	1.5
IGF-1R	0	319	45	14.2	304	38	12.6	
	0.039	1727	99	5.7	1850	126	6.8	0.9
	0.16	5412	194	3.6	4628	60	1.3	1.2
	0.63	16979	843	5.0	14014	702	5.0	1.2
	2.5	57620	1915	3.3	48590	1422	2.9	1.2
	10	170248	5685	3.3	157762	3215	2.0	1.1
IRS-1	0	184	8	4.1	132	8	5.8	
	0.039	191	4	2.1	160	5	3.3	1.2
	0.16	307	17	5.6	200	19	9.7	1.5
	0.63	1311	101	7.7	728	198	27.3	1.8
	2.5	6509	922	14.2	5371	429	8.0	1.2
	10	37077	1182	3.2	26861	3362	12.5	1.4

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. 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.