MSD® Phospho-IRS-1 (Ser312) Assay
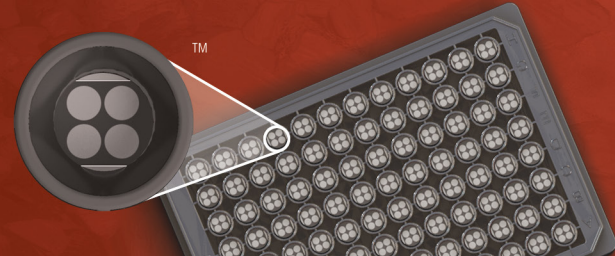# Whole Cell Lysate Kit

For quantitative determination in human whole cell lysate samples

TM

Catalog Numbers

Phospho-IRS-1 (Ser312) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150HLD-1
5 plates	K150HLD-2
20 plates	K150HLD-3

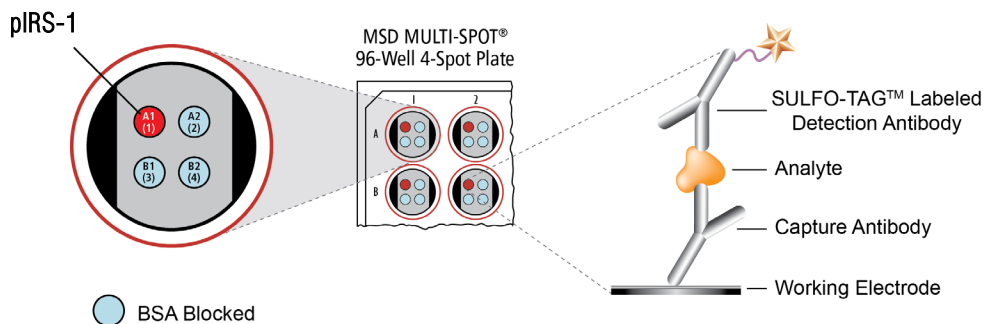| Phospho-IRS-1 (Ser312) Whole Cell Lysate Set	
200 µg	C10HL-1

Ordering information

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

Company Address

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

www.mesoscale.com®

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

pIRS-1

MSD MULTI-SPOT®
96-Well 4-Spot Plate

SULFO-TAG™ Labeled Detection Antibody

Analyte

Capture Antibody

Working Electrode

BSA Blocked

Insulin Receptor Substrate 1 (IRS-1) is a 185 kDa phosphoprotein and a member of the Insulin Receptor Substrate family of proteins. These are cytoplasmic adaptor proteins that propagate a signal from an activated cell surface receptor. IRSs are non-enzymatic and do not directly phosphorylate any other proteins. Their ability to effect downstream signaling is as adaptor proteins which recruit other proteins into a larger signaling complexes.[1] IRS-1 is a signaling intermediate between the Insulin Receptor (IR) and the Insulin like Growth Factor-1 Receptor (IGF-1R) and the downstream PI3K and MAPK signaling cascades. Upon ligand stimulation and autophosphorylation of the IR and IGF-1R, IRS-1 binds to these activated receptors (and receptor hybrids) through its SH2 containing domains, becomes phosphorylated on multiple tyrosine residues, and is able to activate and recruit downstream signaling partners in the PI3K/AKT and ERK/MAPK signaling pathways.[2] The IRS family of proteins are highly studied due to their roles in the very early phases of cancer as well as metastatic progression, and these proteins are seen to have potential both as therapeutic targets of drug development and diagnostic predictors of therapeutic effectiveness, due to their role in drug resistance.[3]

The MSD Phospho-IRS-1 (Ser312) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-IRS-1 (Ser312) 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-IRS-1 (Ser312) and total IRS-1 antibodies and are shown below for comparison.

MCF7 cells were starved in serum-free media for 18 hours (negative) and treated with IGF-1 (100 nM; 20 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total IRS-1 antibody on one of the four spatially distinct electrodes per well. Phosphorylated IRS-1 was detected with anti-phospho-IRS-1 (Ser312) antibody conjugated with MSD SULFO-TAG™ reagent.
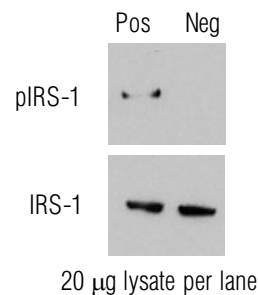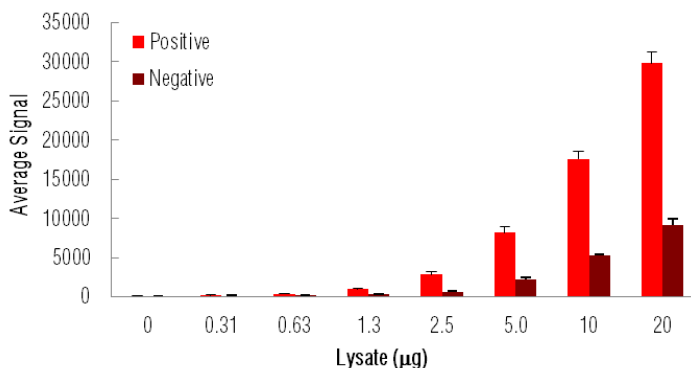
Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-IRS-1 (Ser312) Assay. Increased signal for phosphorylated IRS-1 was observed with pIRS-1 positive cell lysate. The Phospho-IRS-1 (Ser312) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Spot the **Difference**™

MSD®

Lysate Titration

Data for pIRS-1 positive and negative MCF7 cell lysates using the MULTI-ARRAY Phospho-IRS-1 (Ser312) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	64	12	18.2	74	10	13.5	
0.31	191	20	10.5	121	20	16.2	1.6
0.63	373	93	24.8	158	11	6.6	2.4
1.3	988	90	9.1	338	54	16.0	2.9
2.5	2872	378	13.1	632	112	17.6	4.5
5.0	8183	783	9.6	2218	273	12.3	3.7
10	17578	950	5.4	5221	204	3.9	3.4
20	29783	1377	4.6	9110	847	9.3	3.3

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. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature. 1991 Jul 4;352(6330):73-7.
2. Smith TJ. Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? Pharmacol Rev. 2010 Jun;62(2):199-236. Epub 2010 Apr 14.
3. Mardilovich K, Pankratz SL, Shaw LM. Expression and function of the insulin receptor substrate proteins in cancer. Cell Commun Signal. 2009 Jun 17;7:14.