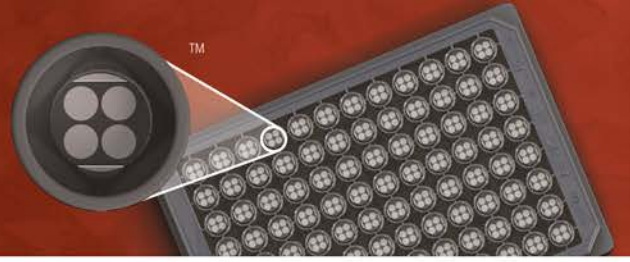
MSD® Phospho-FRS2 (Tyr436) Assay Whole Cell Lysate Kit

For quantitative determination in human and mouse whole cell lysate samples



Alzheimer's Disease
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Catalog Numbers

Phospho-FRS2 (Tyr436) Assay: Whole Cell Lysate Kit	
Kit size	
1 plate	K150KID-1
5 plates	K150KID-2
20 plates	K150KID-3

Phospho-FRS2 (Tyr196/436) Whole Cell Lysate Set	
200 µg	C10KI-1

Ordering information

MSD Customer Service
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Company Address

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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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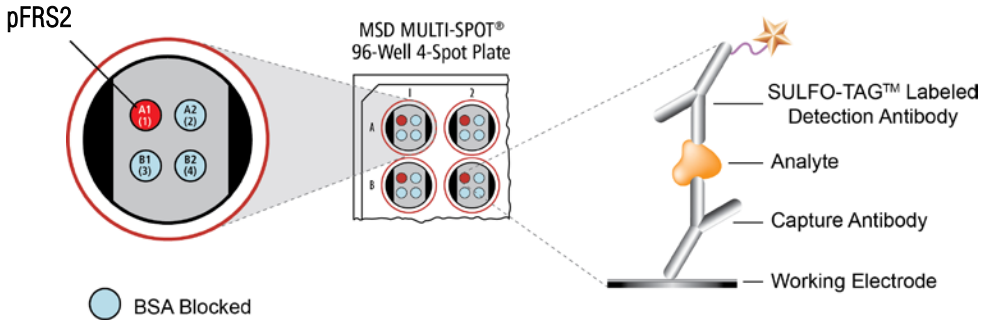

Fibroblast growth factor receptor substrate 2 (FRS2) is a 57 kDa adaptor protein involved in linking the fibroblast and nerve growth factor receptors with the RAS/MAPK signaling pathways. The FRS2 family is comprised of two members, FRS2-α and FRS2-β. Both are composed of an amino terminal myristoylation signal, a phosphotyrosine binding domain, and a carboxy-terminal tail containing adaptor protein binding domains.[1] Within this carboxy terminal tail are two phosphorylation sites: Tyr436, which recruits SHP-2 signaling proteins,[2] and Tyr196, which interacts with GRB-SOS signaling complexes.[2,3] The phosphotyrosine binding domains of FRS2-α and β interact directly with fibroblast and nerve growth factor receptors in both a phosphorylation-dependent and -independent manner.[1] When the FGF receptor binds ligand, it autophosphorylates; this in turn leads to FRS2 binding and subsequent phosphorylation and recruitment of SHP2 and GRB2. This complex binds GAB1, activates PI3K, and converts PIP2 to PIP3 resulting in AKT translocation to the plasma membrane and subsequent downstream signaling through GSK-3β, FOX01, and FOX03.[1-3] The FGF/FGF receptor signaling cascade is believed to play a role in many different types of human cancers, and drugs to modulate this signaling cascade are an active area of pharmaceutical research.[4]

The MSD Phospho-FRS2 (Tyr436) Assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-FRS2 (Tyr436) Assay are illustrated below. The signal and ratio values provided are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-FRS2 (Tyr436) and total FRS2 antibodies and are shown for comparison.
Serum deprived NIH3T3 cells (negative) were treated with FGF (100 ng/mL; 5 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-spot plates coated with anti-total FRS2 antibody on one of the four spatially distinct electrodes within a well. Phosphorylated FRS2 was detected with anti-phospho-FRS2 (Tyr436) antibody conjugated with MSD SULFO-TAG™.

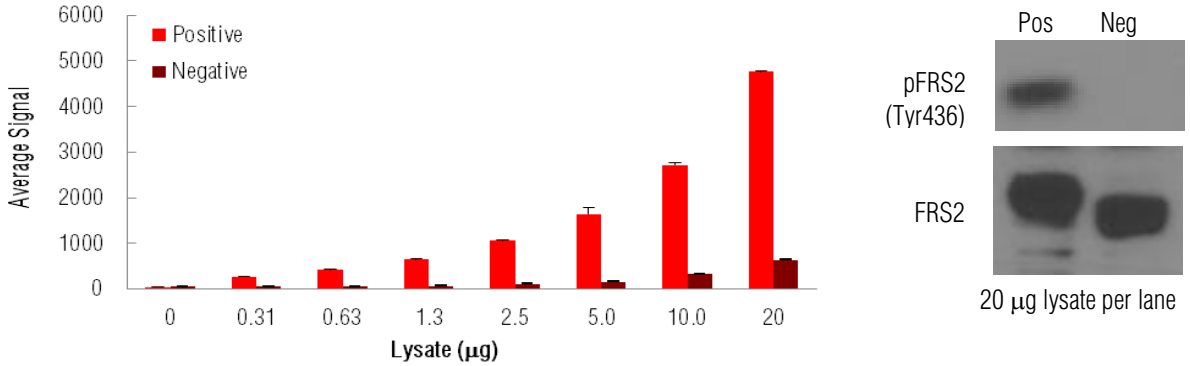

Fig. 1: Sample data generated with the MULTI-ARRAY® Phospho-FRS2 (Tyr436) Assay. Increased signal for phosphorylated FRS2 was observed with pFRS2 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-FRS2 (Tyr436) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for pFRS2 positive and negative NIH3T3 cell lysates using the MULTI-ARRAY Phospho-FRS2 (Tyr436) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	37	0	0.0	54	1	2.6	
0.31	274	11	3.9	51	6	11.1	5.4
0.63	417	16	3.9	60	6	10.7	7.0
1.3	646	28	4.3	66	12	18.4	9.9
2.5	1056	9	0.9	105	11	10.8	10
5.0	1646	150	9.1	157	6	3.6	10
10	2712	54	2.0	325	4	1.1	8.4
20	4767	8	0.2	636	13	2.0	7.5

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. Ong SH, Guy GR, Hadari YR, Laks S, Gotoh N, Schlessinger J, Lax I. FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. Mol Cell Biol. 2000 Feb;20(3):979-89.
2. Hadari YR, Gotoh N, Kouhara H, Lax I, Schlessinger J. Critical role for the docking-protein FRS2 alpha in FGF receptor-mediated signal transduction pathways. Proc Natl Acad Sci U S A. 2001 Jul 17;98(15):8578-83.
3. Gotoh N. Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins. Cancer Sci. 2008 Jul;99(7):1319-25.
4. Jeffers M, LaRochelle WJ, Lichenstein HS. Fibroblast growth factors in cancer: therapeutic possibilities. Expert Opin Ther Targets. 2002 Aug;6(4):469-82.