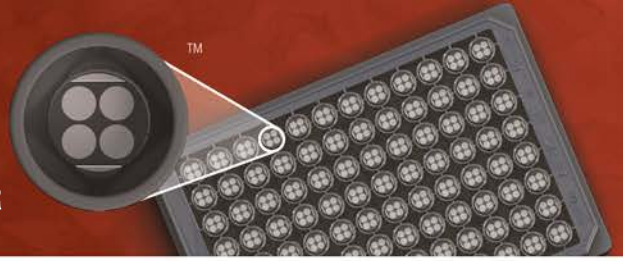
MSD® EGFR Family Whole Cell Lysate Kit

For quantitative determination of phospho-EGFR, phospho-ErbB2, and phospho-IGF-1R 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

EGFR Family Whole Cell Lysate Kit	
Kit size	
1 plate	K15106D-1
5 plates	K15106D-2
20 plates	K15106D-3

Ordering information

MSD Customer Service
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Email: CustomerService@mesoscale.com

Company Address

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Gaithersburg, MD 20877 USA

www.mesoscale.com®

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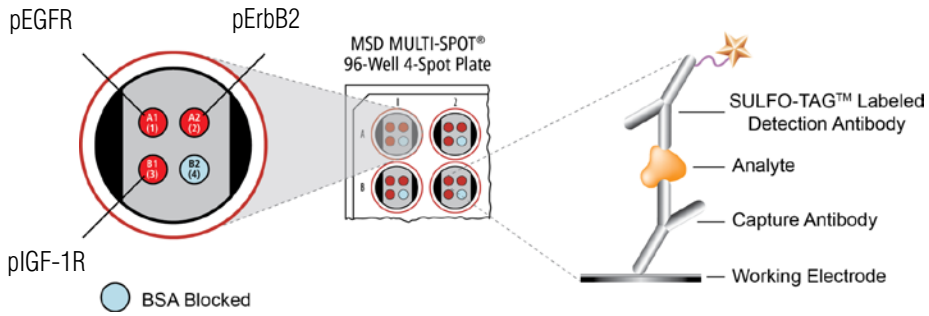
Not for use in diagnostic procedures.

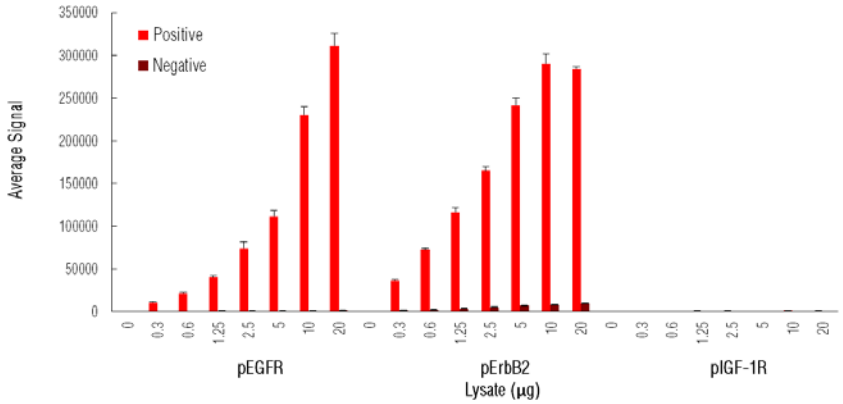


Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor Receptor 2 (Her2/Neu/ErbB2), and Insulin like Growth Factor I Receptor (IGF-1R) are all receptor tyrosine kinases with extracellular ligand binding domains and intracellular tyrosine kinase domains. In normal cells these receptors play key roles in growth and development whereas IGF-1R also plays a critical role in metabolism and determination of overall nutrient state of the organism. All three of these receptors also signal through the PI3K-Akt signaling pathway and play a role in both normal as well as cancer cell development. EGFR and ErbB2 are oncoproteins and are involved in carcinogenesis, tumor growth/progression, and metastasis.[1] Many different cancers (such as breast cancer, prostate cancer, GI cancers, ovarian, neuroblastoma, and others) show increased expression of IGF-1R as well as IGF-1 and the level of expression tracks with tumor progression and cancer stage.[2] EGFR and ErbB2 are also involved in many different types of cancers, such as head, neck, colon, breast, pancreas, and lung cancers.[3] These important intracellular signaling receptors have been the subject of many basic research and drug development programs.

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

Typical Data with SKOV3 Cells: Control Lysates for Phospho-EGFR and Phospho-ErbB2

Representative results for the EGFR Family are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Serum deprived SKOV3 cells were treated with Compound 56 and AG825 (1 μM each, 2.5 hours) (negative) or pretreated with sodium vanadate (1 mM, 4 hours) and stimulated with EGF (100 ng/mL, 10 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-total EGFR, anti-total ErbB2, and anti-total IGF-1R antibodies on three of the four spatially distinct electrodes within a well. Phosphorylated EGFR, ErbB2, and IGF-1R were detected with an anti-phosphotyrosine antibody conjugated with MSD SULFO-TAG™ reagent. Western blot analyses of each lysate type were performed with phospho-EGFR, phospho-ErbB2, and phospho-IGF-1R antibodies and are shown below for comparison.



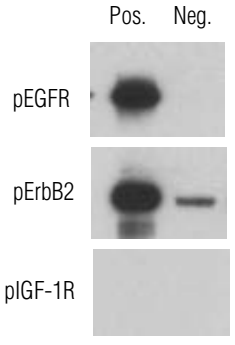


Fig. 1: Sample data generated with MULTI-SPOT EGFR Family. Increased signals for pEGFR and pErbB2 were observed with only the positive cell lysate. Signals for phospho-IGF-1R remained low throughout the titration of positive lysate. Signals for negative lysate were also low throughout the titration for all assays.

Lysate Titration

Data for positive and negative SKOV3 cell lysates using the MULTI-SPOT EGFR Family are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pEGFR	0	81	26	32.1	74	13	17.2	
	0.31	10782	779	7.2	141	27	19.4	76
	0.63	21653	981	4.5	195	9	4.6	111
	1.3	40238	2499	6.2	239	13	5.6	169
	2.5	74001	7543	10.2	295	16	5.4	251
	5.0	111613	6754	6.1	348	34	9.7	321
	10	230708	8906	3.9	706	63	8.9	327
	20	311189	14667	4.7	1166	183	15.7	267
pErbB2	0	139	29	21.2	123	17	13.9	
	0.31	36591	1301	3.6	1152	52	4.5	32
	0.63	72742	1514	2.1	2117	46	2.1	34
	1.3	116381	5075	4.4	3448	130	3.8	34
	2.5	165277	5082	3.1	4818	413	8.6	34
	5.0	241421	8847	3.7	7184	231	3.2	34
	10	290486	11243	3.9	8151	255	3.1	36
	20	284703	2170	0.8	9648	110	1.1	30
pIGF-1R	0	93	8	8.2	103	16	15.7	
	0.31	503	60	11.9	131	21	15.8	3.8
	0.63	614	24	3.9	154	6	3.8	4.0
	1.3	784	18	2.3	167	5	3.0	4.7
	2.5	963	81	8.5	167	12	7.3	5.8
	5.0	592	36	6.1	133	40	29.8	4.4
	10	1102	29	2.6	181	4	2.2	6.1
	20	813	53	6.5	193	17	8.8	4.2

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Typical Data with A431 Cells: Control Lysates for Phospho-IGF-1R

Representative results for the EGFR Family are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Serum deprived A431 cells (negative) were pretreated with sodium vanadate (1 mM, 4 hours) and stimulated with IGF-1 (100 nM, 10 minutes) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-total EGFR, anti-total ErbB2, and anti-total IGF-1R antibodies on three of the four spatially distinct electrodes per well. Phosphorylated EGFR, ErbB2, and IGF-1R were detected with anti-phosphotyrosine antibody conjugated with MSD SULFO-TAG reagent. Western blot analyses of each lysate type were performed with phospho-EGFR, phospho-ErbB2, and phospho-IGF-1R antibodies and are shown below for comparison.

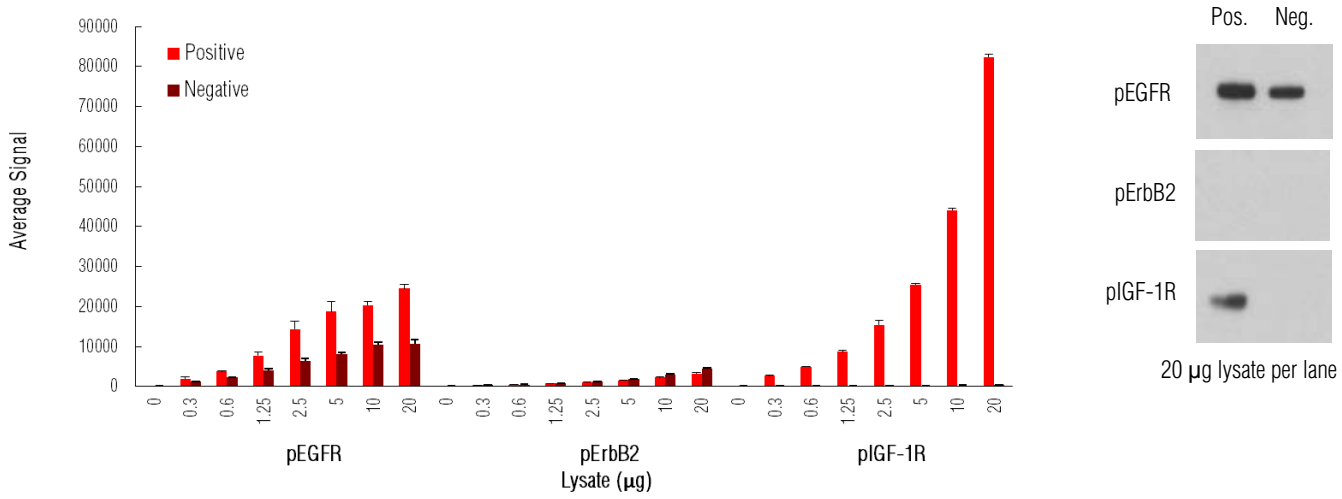

Fig. 2: Sample data generated with MULTI-SPOT EGFR Family. Increased signals for pEGFR and pIGF-1R were observed with the positive cell lysate. Signals for phospho-ErbB2 were relatively low.

Lysate Titration

Data for positive and negative A431 cell lysates using the MULTI-SPOT EGFR Family are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pEGFR	0	72	7	10.0	70	15	20.9	
	0.31	1933	406	21.0	1174	35	3.0	1.6
	0.63	3720	129	3.5	2289	80	3.5	1.6
	1.3	7733	907	11.7	3972	462	11.6	1.9
	2.5	14358	2046	14.2	6346	672	10.6	2.3
	5.0	18697	2466	13.2	8113	471	5.8	2.3
	10	20289	1016	5.0	10537	627	5.9	1.9
	20	24540	1068	4.4	10558	1230	11.6	2.3

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pErbB2	0	110	13	12.1	111	1	1.0	
	0.31	297	23	7.7	312	19	6.1	0.9
	0.63	480	15	3.1	483	29	5.9	1.0
	1.3	729	22	3.0	740	17	2.3	1.0
	2.5	1027	54	5.2	1177	29	2.4	0.9
	5.0	1500	95	6.4	1807	20	1.1	0.8
	10	2254	96	4.3	2970	122	4.1	0.8
	20	3228	226	7.0	4506	231	5.1	0.7
pIGF-1R	0	109	7	6.3	93	5	5.4	
	0.31	2682	178	6.6	152	12	7.7	18
	0.63	4975	82	1.6	165	4	2.6	30
	1.3	8623	373	4.3	196	10	4.8	44
	2.5	15432	1163	7.5	228	7	3.1	68
	5.0	25444	297	1.2	222	22	9.7	115
	10	44107	563	1.3	267	11	4.1	165
	20	82362	649	0.8	299	21	7.0	276

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. Gazdar AF. Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene. 2009 Aug;28 Suppl 1:S24-31.
2. Li R, Pourpak A, Morris SW. Inhibition of the insulin-like growth factor-1 receptor (IGF-1R) tyrosine kinase as a novel cancer therapy approach. J Med Chem. 2009 Aug 27;52(16):4981-5004.
3. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors—impact on future treatment strategies. Nat Rev Clin Oncol. 2010 Sep;7(9):493-507. Epub 2010 Jun 15.