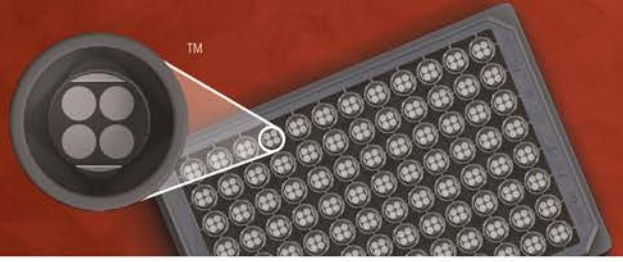
MSD® Total EGFR Assay Whole Cell Lysate Kit

For quantitative determination 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

Total EGFR Whole Cell Lysate Kit	
Kit size	
1 plate	K151CKD-1
5 plates	K151CKD-2
20 plates	K151CKD-3

Phospho-EGFR Whole Cell Lysate Set	
200 µg	C11CI-1

Ordering information

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

Company Address

MESO SCALE DISCOVERY®
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Gaithersburg, MD 20877 USA

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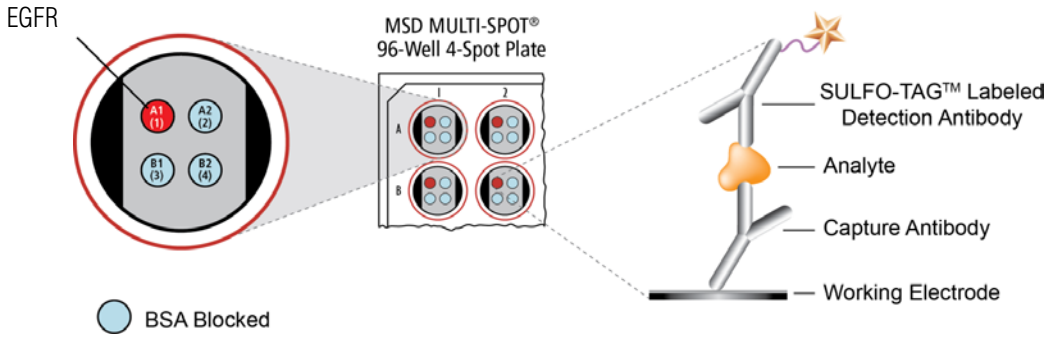

EGFR (Epidermal Growth Factor Receptor) is a 170 kDa transmembrane receptor tyrosine kinase, consisting of a ligand-binding extracellular domain, a single transmembrane domain, an intracellular protein-tyrosine kinase catalytic domain, and a tyrosine-containing cytoplasmic tail. EGFR (ErbB1/HER1) is one of a family of four ErbB/HER (1-4) receptor tyrosine kinases, each essential to embryonic survival. Upon binding its ligand EGF, the EGFR forms hetero- or homodimers. Dimerization results in the activation of its intrinsic tyrosine kinase activity and the phosphorylation of multiple tyrosines in the cytoplasmic domain, including Tyr992, Tyr1068, Tyr1086, Tyr1148, and Tyr1173. The phosphorylated tyrosines are binding sites for proteins containing SH2-domains. These binding events activate many intracellular signaling pathways including MAPK/ERK, PI-3K, PKC, and p38, controlling cell growth, survival, cell cycle arrest, and transformation. Due to its central role in many cellular physiological processes, EGFR overexpression and aberrant signaling is associated with many types of cancer, making EGFR an attractive target for chemotherapeutic drug development

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

Typical Data with A431 cells treated with EGF or Compound 56

Representative results for the Total EGFR Assay 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 were treated with compound 56 (5 nM; 3 hours) (negative), or 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 antibody on one of the four spatially distinct electrodes per well. Total EGFR was detected with anti-total EGFR antibody conjugated with MSD SULFO-TAG™ reagent. Western blot analysis of each lysate type was performed with total EGFR antibody and is shown below for comparison.

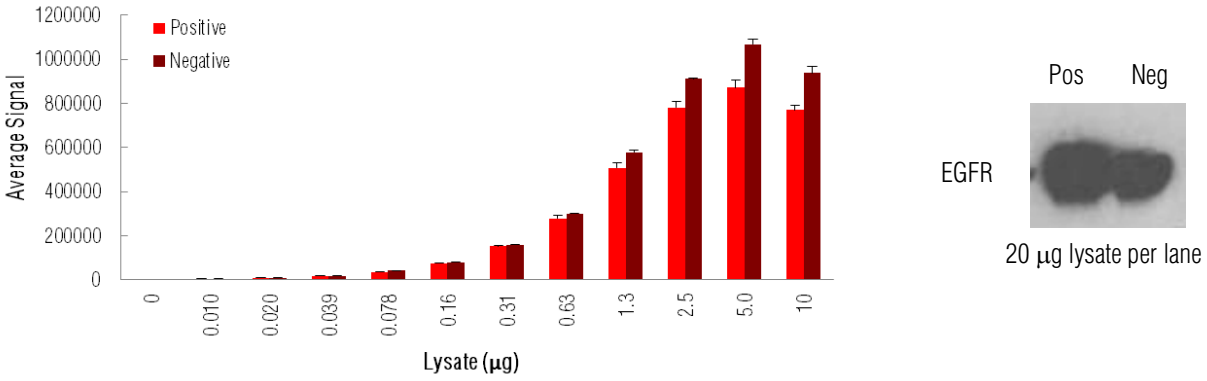

Fig. 1: Sample data generated with MULTI-ARRAY® Total EGFR Assay. Increased signal is observed with the titration of both pEGFR positive and negative cell lysates. The Total EGFR Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative A431 cell lysates using the MULTI-ARRAY Total EGFR Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	110	11	9.7	110	11	9.7	
0.010	4839	535	11.1	4879	55	1.1	1.0
0.020	9091	900	9.9	9406	51	0.5	1.0
0.039	18011	1604	8.9	19178	677	3.5	0.9
0.078	36092	1296	3.6	41489	532	1.3	0.9
0.16	75165	1672	2.2	79915	1241	1.6	0.9
0.31	151563	4679	3.1	158189	370	0.2	1.0
0.63	279325	14269	5.1	300471	1910	0.6	0.9
1.3	508096	22131	4.4	577715	9687	1.7	0.9
2.5	781728	25990	3.3	910844	3020	0.3	0.9
5.0	871694	33787	3.9	1067114	25666	2.4	0.8
10	769647	20999	2.7	936608	30138	3.2	0.8

Typical Data with COS7 cells treated with EGF

Representative results for the Total EGFR Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Serum deprived COS-7 cells (negative) were treated 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 antibody on one of the four spatially distinct electrodes per well. Total EGFR was detected with anti-total EGFR antibody conjugated with MSD SULFO-TAG reagent. Western blot analysis of each lysate type was performed with total EGFR antibody and is shown below for comparison.

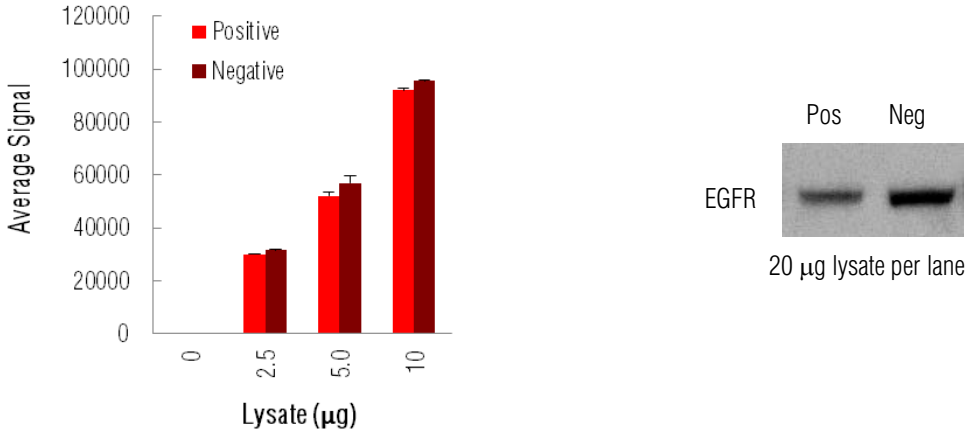

Fig. 2: Sample data generated with MULTI-ARRAY Total EGFR Assay. Increased signal is observed with the titration of both pEGFR positive and negative cell lysates. The Total EGFR Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative COS7 cell lysates using the MULTI-ARRAY Total EGFR are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	146	2	1.5	146	2	1.5	
2.5	30050	33	0.1	31626	445	1.4	1.0
5.0	51992	1493	2.9	57029	2446	4.3	0.9
10	91971	940	1.0	95429	257	0.3	1.0

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

For a complete list of products, please visit our website at www.mesoscale.com

References using MSD's technology for the measurement of phosphoproteins

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2. Cao L, Yu Y, Darko I, Currier D, Mayeenuddin LH, Wan X, Khanna C, Helman LJ. Addiction to elevated insulin-like growth factor I receptor and initial modulation of the AKT pathway define the responsiveness of rhabdomyosarcoma to the targeting antibody. Cancer Res. 2008 Oct 1;68(19):8039-48.
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