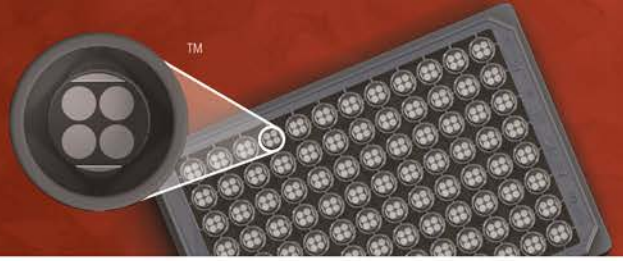
MSD® Phospho-EGFR (Tyr1173) Assay Whole Cell Lysate Kit

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

Phospho-EGFR (Tyr1173) Whole Cell Lysate Kit	
Kit size	
1 plate	K151CJD-1
5 plates	K151CJD-2
20 plates	K151CJD-3

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

Ordering information

MSD Customer Service
Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Company Address

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

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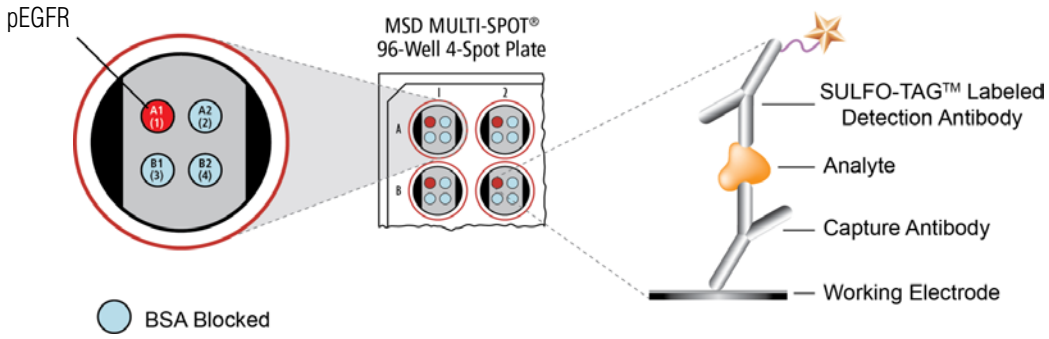

EGFR (Epidermal Growth Factor Receptor) is a 170 kDa transmembrane receptor tyrosine kinase, consisting of a ligand-binding extracellular domain, a single transmembrane domain, an intracellular protein-tyrosine kinase catalytic domain, and a tyrosine-containing cytoplasmic tail. EGFR (ErbB1/HER1) is 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 Phospho-EGFR (Tyr1173) 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 Phospho-EGFR (Tyr1173) 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-phospho-EGFR antibody on one of the four spatially distinct electrodes per well. Phosphorylated EGFR was detected with anti-total EGFR antibody conjugated with MSD SULFO-TAG™ reagent. Western blot analyses of each lysate type were performed with phospho-EGFR (Tyr1173) and total EGFR antibodies and are shown below for comparison.

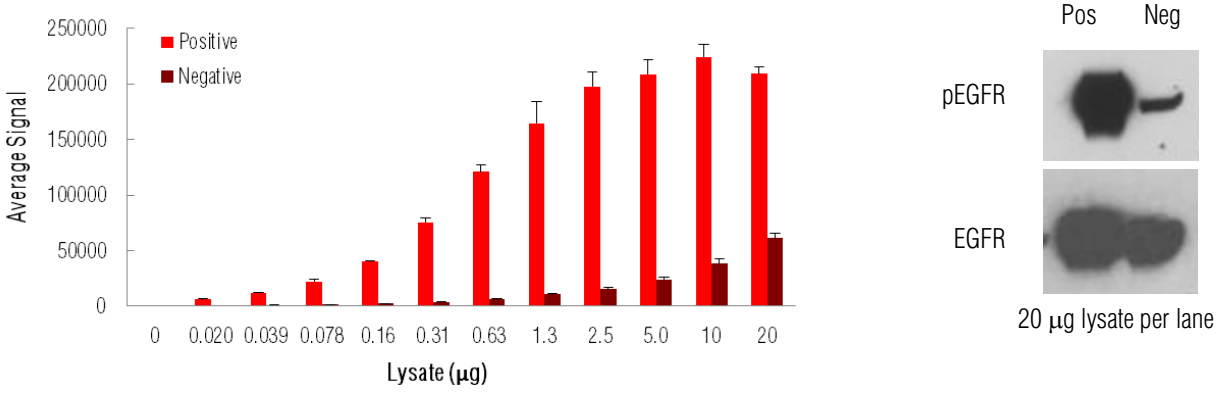

Fig. 1: Sample data generated with MULTI-ARRAY® Phospho-EGFR (Tyr1173) Assay. Increased signal is observed with the titration of pEGFR positive cell lysate. The Phospho-EGFR (Tyr1173) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative A431 cell lysates using the MULTI-ARRAY Phospho-EGFR (Tyr1173) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	63	12	19.2	63	12	19.2	
0.020	5887	666	11.3	451	17	3.8	13
0.039	11918	601	5.0	766	60	7.8	16
0.078	22373	1596	7.1	1298	26	2.0	17
0.16	40530	576	1.4	2241	224	10.0	18
0.31	75651	3478	4.6	3842	249	6.5	20
0.63	120833	6403	5.3	6117	311	5.1	20
1.3	164670	18997	11.5	10542	450	4.3	16
2.5	197830	12610	6.4	15451	1227	7.9	13
5.0	208587	12978	6.2	23947	2028	8.5	8.7
10	224371	11497	5.1	38414	4128	10.7	5.8
20	209044	6404	3.1	61388	3803	6.2	3.4

Typical Data with COS7 cells treated with EGF

Representative results for the Phospho-EGFR (Tyr1173) 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-phospho-EGFR antibody on one of the four spatially distinct electrodes per well. Phosphorylated EGFR was detected with anti-total EGFR antibody conjugated with MSD SULFO-TAG reagent. Western blot analyses of each lysate type were performed with phospho-EGFR (Tyr1173) and total EGFR antibodies and are shown below for comparison.

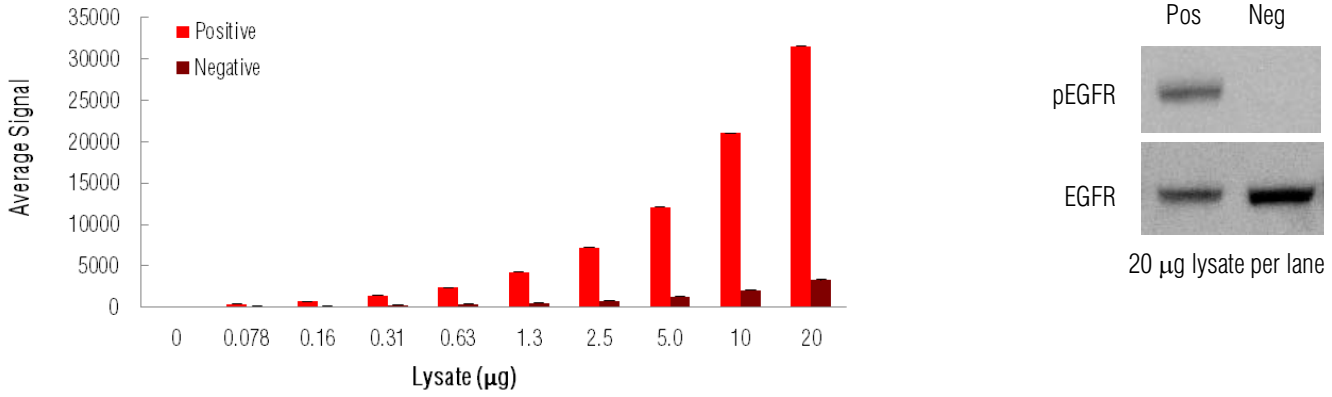

Fig. 2: Sample data generated with MULTI-ARRAY Phospho-EGFR (Tyr1173) Assay. Increased signal is observed with the titration of pEGFR positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-EGFR (Tyr1173) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

Lysate Titration

Data for positive and negative COS7 cell lysates using the MULTI-ARRAY Phospho-EGFR (Tyr1173) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	70	25	35.5	70	25	35.5	
0.078	423	22	5.2	103	13	12.7	4.1
0.16	745	17	2.3	145	25	17.5	5.1
0.31	1403	40	2.8	261	5	2.0	5.4
0.63	2420	53	2.2	378	30	8.0	6.4
1.3	4243	439	10.3	551	43	7.9	7.7
2.5	7237	182	2.5	811	15	1.9	8.9
5.0	12119	454	3.7	1329	47	3.6	9.1
10	21027	339	1.6	2055	93	4.5	10
20	31519	1130	3.6	3281	130	3.9	9.6

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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4. Gowan SM, Hardcastle A, Hallsworth AE, Valenti MR, Hunter LJ, de Haven Brandon AK, Garrett MD, Raynaud F, Workman P, Aherne W, Eccles SA. Application of meso scale technology for the measurement of phosphoproteins in human tumor xenografts. Assay Drug Dev Technol. 2007 Jun;5(3):391-401.