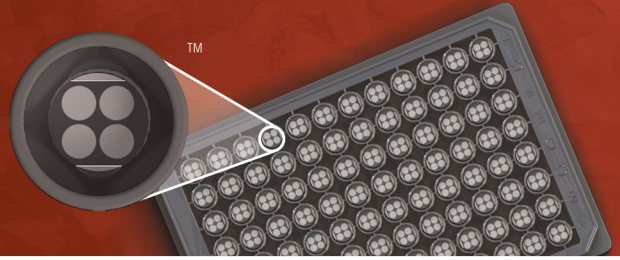
# MSD® Phospho(Tyr1173)/Total EGFR Assay Whole Cell Lysate Kit

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

| Phospho (Tyr1173)/Total EGFR Whole Cell Lysate Kit | |
|---|---|
| Kit size | |
| 1 plate | K15104D-1 |
| 5 plates | K15104D-2 |
| 20 plates | K15104D-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

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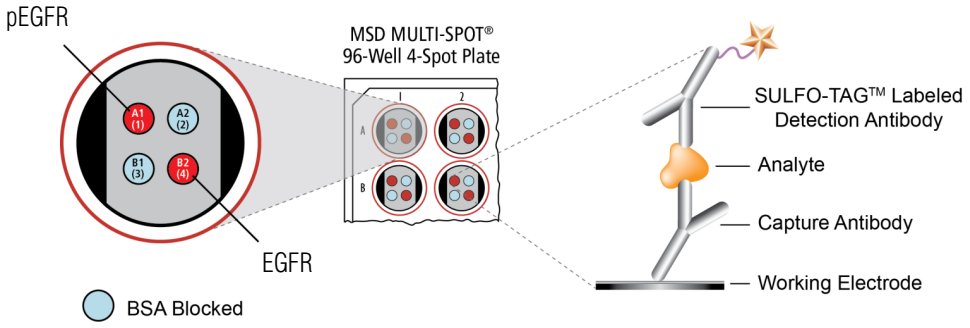


**EGFR (Epidermal Growth Factor Receptor)** is a 170 kDa transmembrane receptor tyrosine kinase, consisting of a ligand-binding extracellular domain, a single transmembrane domain, an intracellular protein-tyrosine kinase catalytic domain, and a tyrosine-containing cytoplasmic tail. EGFR (ErbB1/HER1) 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(Tyr1173)/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 Phospho(Tyr1173)/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-phospho-EGFR (Tyr1173) and anti-total EGFR antibodies on spatially distinct electrodes within a well. Phosphorylated and total EGFR were 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.

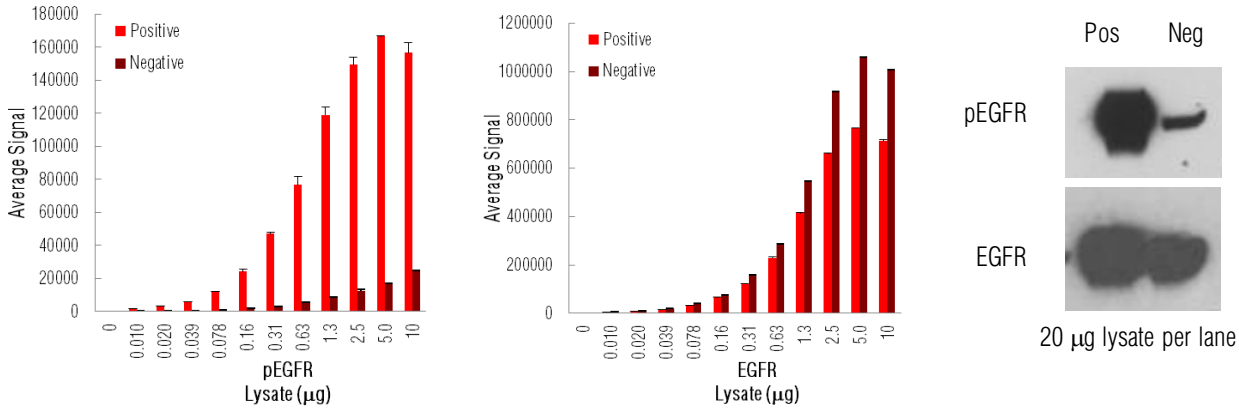


**Fig. 1:** Sample data generated with MULTI-SPOT Phospho(Tyr1173)/Total EGFR Assay. Increased signal for phosphorylated EGFR was observed with pEGFR positive cell lysate. Total EGFR signal increased throughout the titration of both pEGFR positive and negative cell lysates. The Phospho(Tyr1173)/Total EGFR Assay provides a quantitative measure of the data obtained with the traditional Western blot.

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

Spot the Difference™

## Lysate Titration

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

| | Lysate (µg) | Positive | | | Negative | | | P/N |
|---|---|---|---|---|---|---|---|---|
| | | Average Signal | StdDev | %CV | Average Signal | StdDev | %CV | |
| pEGFR | 0 | 83 | 37 | 45.4 | 83 | 37 | 45.4 | |
| | 0.010 | 1535 | 28 | 1.8 | 149 | 2 | 1.4 | 10 |
| | 0.020 | 3093 | 272 | 8.8 | 242 | 11 | 4.7 | 13 |
| | 0.039 | 5902 | 12 | 0.2 | 445 | 46 | 10.3 | 13 |
| | 0.078 | 11857 | 193 | 1.6 | 913 | 53 | 5.8 | 13 |
| | 0.16 | 24147 | 1647 | 6.8 | 1688 | 121 | 7.2 | 14 |
| | 0.31 | 47152 | 573 | 1.2 | 3174 | 29 | 0.9 | 15 |
| | 0.63 | 76705 | 4835 | 6.3 | 5215 | 573 | 11.0 | 15 |
| | 1.3 | 118913 | 4691 | 3.9 | 8261 | 431 | 5.2 | 14 |
| | 2.5 | 149480 | 4329 | 2.9 | 12501 | 725 | 5.8 | 12 |
| | 5.0 | 166478 | 311 | 0.2 | 16809 | 272 | 1.6 | 9.9 |
| | 10 | 156688 | 6253 | 4.0 | 24320 | 398 | 1.6 | 6.4 |
| EGFR | 0 | 184 | 52 | 28.1 | 184 | 52 | 28.1 | |
| | 0.010 | 3861 | 18 | 0.5 | 4958 | 325 | 6.5 | 0.8 |
| | 0.020 | 7637 | 180 | 2.4 | 9914 | 670 | 6.8 | 0.8 |
| | 0.039 | 15669 | 236 | 1.5 | 18656 | 1650 | 8.8 | 0.8 |
| | 0.078 | 30948 | 817 | 2.6 | 39656 | 276 | 0.7 | 0.8 |
| | 0.16 | 64429 | 4687 | 7.3 | 76377 | 2477 | 3.2 | 0.8 |
| | 0.31 | 121570 | 292 | 0.2 | 157668 | 5238 | 3.3 | 0.8 |
| | 0.63 | 228249 | 9044 | 4.0 | 285751 | 19146 | 6.7 | 0.8 |
| | 1.3 | 413168 | 675 | 0.2 | 544371 | 2655 | 0.5 | 0.8 |
| | 2.5 | 659744 | 7092 | 1.1 | 914856 | 23540 | 2.6 | 0.7 |
| | 5.0 | 766507 | 63037 | 8.2 | 1058852 | 3263 | 0.3 | 0.7 |
| | 10 | 711199 | 1256 | 0.2 | 1006322 | 14843 | 1.5 | 0.7 |

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## Typical Data with COS7 cells treated with EGF

Representative results for the Phospho(Tyr1173)/Total EGFR 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 (Tyr1173) and anti-total EGFR antibodies on spatially distinct electrodes within a well. Phosphorylated and total EGFR were 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.

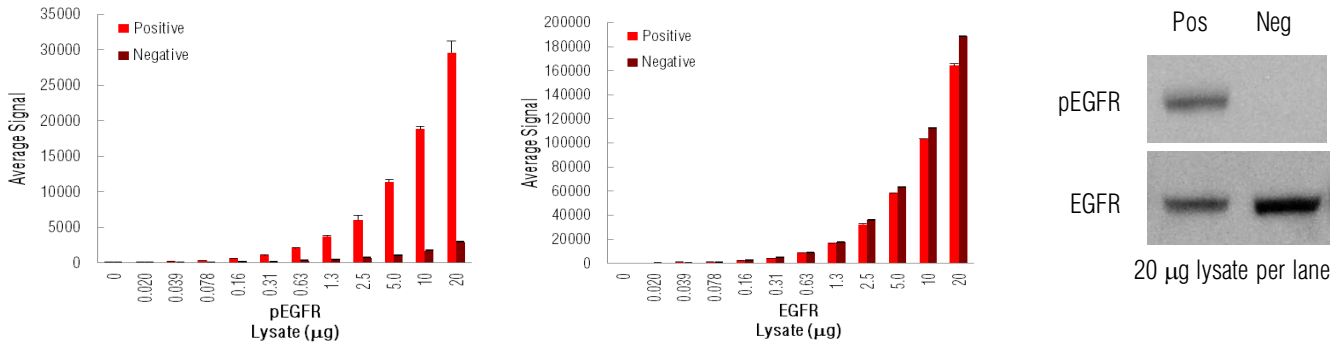


**Fig. 2:** Sample data generated with MULTI-ARRAY Phospho-EGFR (Tyr1173) Assay. Increased signal for phosphorylated EGFR was observed with pEGFR positive cell lysate. Total EGFR signal increased throughout the titration of both pEGFR positive and negative cell lysates. The Phospho(Tyr1173)/Total EGFR 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-SPOT Phospho(Tyr1173)/Total EGFR are presented below.

| | Lysate (µg) | Positive | | | Negative | | | P/N |
|---|---|---|---|---|---|---|---|---|
| | | Average Signal | StdDev | %CV | Average Signal | StdDev | %CV | |
| pEGFR | 0 | 74 | 13 | 17.2 | 74 | 13 | 17.2 | |
| | 0.020 | 124 | 12 | 9.7 | 74 | 1 | 1.9 | 1.7 |
| | 0.039 | 226 | 23 | 10.3 | 81 | 8 | 9.7 | 2.8 |
| | 0.078 | 319 | 19 | 6.0 | 95 | 8 | 8.2 | 3.4 |
| | 0.16 | 606 | 6 | 0.9 | 161 | 6 | 4.0 | 3.8 |
| | 0.31 | 1083 | 44 | 4.0 | 214 | 5 | 2.3 | 5.1 |
| | 0.63 | 2068 | 119 | 5.7 | 311 | 32 | 10.2 | 6.7 |
| | 1.3 | 3742 | 95 | 2.6 | 424 | 8 | 2.0 | 8.8 |
| | 2.5 | 5986 | 717 | 12.0 | 705 | 40 | 5.6 | 8.5 |
| | 5.0 | 11415 | 305 | 2.7 | 1098 | 21 | 1.9 | 10 |
| | 10 | 18828 | 373 | 2.0 | 1712 | 34 | 2.0 | 11 |
| | 20 | 29591 | 1651 | 5.6 | 2935 | 38 | 1.3 | 10 |

| | Lysate (µg) | Positive | | | Negative | | | P/N |
|---|---|---|---|---|---|---|---|---|
| | | Average Signal | StdDev | %CV | Average Signal | StdDev | %CV | |
| EGFR | 0 | 117 | 17 | 14.5 | 117 | 17 | 14.5 | |
| | 0.020 | 382 | 1 | 0.4 | 431 | 38 | 8.9 | 0.9 |
| | 0.039 | 1046 | 536 | 51.2 | 700 | 9 | 1.3 | 1.5 |
| | 0.078 | 1115 | 81 | 7.2 | 1268 | 59 | 4.6 | 0.9 |
| | 0.16 | 2199 | 111 | 5.0 | 2489 | 115 | 4.6 | 0.9 |
| | 0.31 | 4421 | 203 | 4.6 | 4866 | 172 | 3.5 | 0.9 |
| | 0.63 | 8729 | 93 | 1.1 | 9086 | 222 | 2.4 | 1.0 |
| | 1.3 | 16657 | 489 | 2.9 | 17907 | 643 | 3.6 | 0.9 |
| | 2.5 | 32427 | 1176 | 3.6 | 35960 | 1232 | 3.4 | 0.9 |
| | 5.0 | 58184 | 924 | 1.6 | 63150 | 2567 | 4.1 | 0.9 |
| | 10 | 103256 | 4692 | 4.5 | 112130 | 465 | 0.4 | 0.9 |
| | 20 | 164260 | 7383 | 4.5 | 188770 | 4751 | 2.5 | 0.9 |

## 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. Rogers SJ, Box C, Chambers P, Barbachano Y, Nutting CM, Rhŷs-Evans P, Workman P, Harrington KJ, Eccles SA. Determinants of response to epidermal growth factor receptor tyrosine kinase inhibition in squamous cell carcinoma of the head and neck. J Pathol. 2009 May;218(1):122-30.
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.
3. Martin SE, Jones TL, Thomas CL, Lorenzi PL, Nguyen DA, Runfola T, Gunsior M, Weinstein JN, Goldsmith PK, Lader E, Huppi K, Caplen NJ. Multiplexing siRNAs to compress RNAi-based screen size in human cells. Nucleic Acids Res. 2007;35(8):e57. Epub 2007 Mar 28.
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