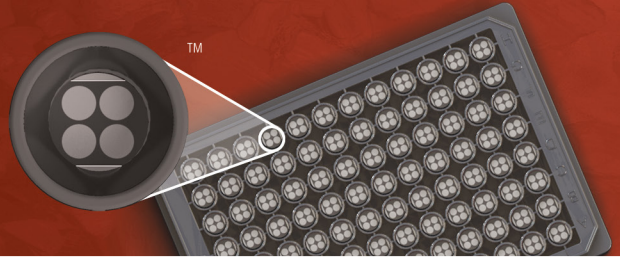
MSD® Phospho-EGFR (Tyr1068) Custom Assay Whole Cell Lysate Kit

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 Whole Cell Lysate Set	
200 µg	C11CI-1

Ordering information

MSD Customer Service
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Email: CustomerService@mesoscale.com

Company Address

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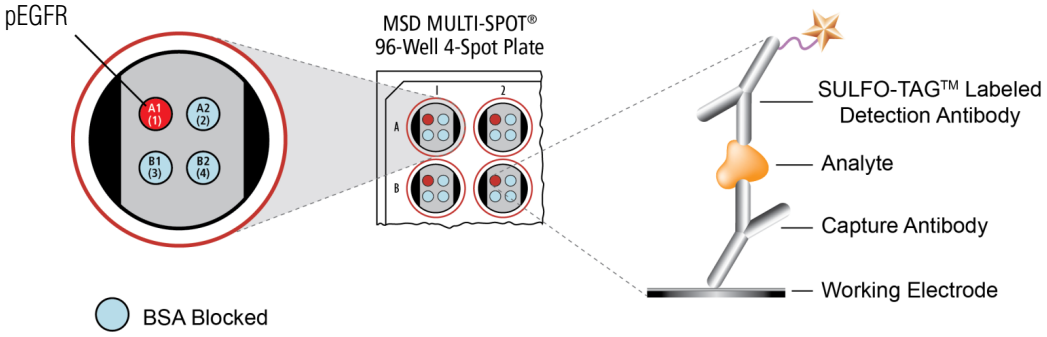
www.mesoscale.com®



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 (Tyr1068) Assay is available on 96-well 4-spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho-EGFR (Tyr1068) 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 (1 µM; 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™. Western blot analyses of each lysate type were performed with phospho-EGFR (Tyr1068) and total EGFR antibodies and are shown below for comparison.

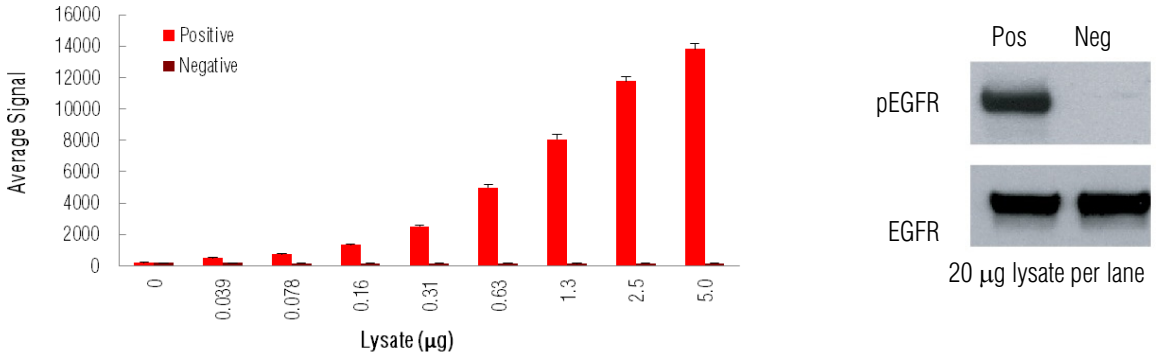


Fig. 1: Sample data generated with MULTI-ARRAY® Phospho-EGFR (Tyr1068) Assay. Increased signal is observed with the titration of pEGFR positive cell lysate. The Phospho-EGFR (Tyr1068) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

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

Lysate Titration

Data for positive and negative A431 cell lysates using the MULTI-ARRAY Phospho-EGFR (Tyr1068) Assay are presented below.

Lysate (µg)	Positive			Negative			P/N
	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
0	192	14	7.3	184	15	8.2	
0.039	494	45	9.1	182	10	5.5	2.7
0.078	723	61	8.4	170	28	16.5	4.3
0.16	1345	28	2.1	171	7	4.1	7.9
0.31	2499	95	3.8	150	14	9.3	17
0.63	4977	184	3.7	173	16	9.2	29
1.3	8058	309	3.8	143	13	9.1	56
2.5	11776	265	2.3	148	13	8.8	80
5.0	13808	378	2.7	168	16	9.5	82

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

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. 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.