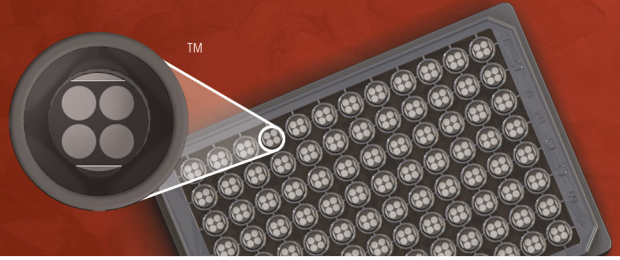
MSD® Phospho(Tyr1248)/Total ErbB2 Assay Whole Cell Lysate Kit

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 (Tyr1248)/Total ErbB2 Whole Cell Lysate Kit	
Kit size	
1 plate	K15125D-1
5 plates	K15125D-2
20 plates	K15125D-3

Ordering information

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

Company Address

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A division of
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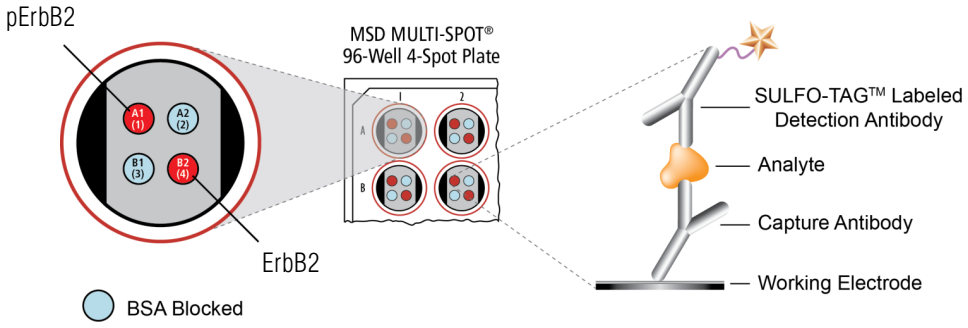
Gaithersburg, MD 20877 USA

www.mesoscale.com®



ErbB2 (HER-2/Neu) is a 185 kDa member belonging to a family of four type I receptor-tyrosine kinases structurally consisting of an ectodomain, a single transmembrane segment, and a cytoplasmic region including a protein tyrosine kinase domain. ErbB2 currently does not have any known direct ligand, but becomes activated through overexpression or heterodimerization with other members of the ErbB family. Activation of ErbB2 results in the autophosphorylation of several tyrosines (including 1248) on the intracellular domain of the receptor. This tyrosine phosphorylation links ErbB2 with several intracellular signaling pathways including the PI-3 kinase, ERK, and p38 pathways through proteins such as Ras, Grb2, and Shc. ErbB2 activation exerts an effect on a variety of cellular processes including transformation, proliferation and survival, apoptosis, and development. Overexpression of ErbB2 has been detected in several types of human cancers including breast, ovarian, and prostate, and its overexpression alone, as well as coupled with p53 accumulation and the cytoplasmic location of p21, is correlated with a poor breast cancer patient prognosis. ErbB2 is a major target of anti-cancer drugs, with the ectodomain-directed antibody Herceptin currently approved for breast cancer treatment.

The MSD Phospho(Tyr1248)/Total ErbB2 Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

Typical Data

Representative results for the Phospho(Tyr1248)/Total ErbB2 Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Western blot analyses of each lysate type were performed with phospho-ErbB2 (Tyr1248) and total ErbB2 antibodies and are shown below for comparison. Serum deprived SK-OV3 cells were treated with sodium vanadate (1 mM; 4 hours) followed by EGF stimulation (100 ng/mL; 10 minutes) (positive) or Compound 56 and AG825 (1 μM; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-ErbB2 (Tyr1248) and anti-total ErbB2 antibodies on spatially distinct electrodes within a well. Phosphorylated and total ErbB2 were detected with anti-total ErbB2 antibody conjugated with MSD SULFO-TAG™ reagent.

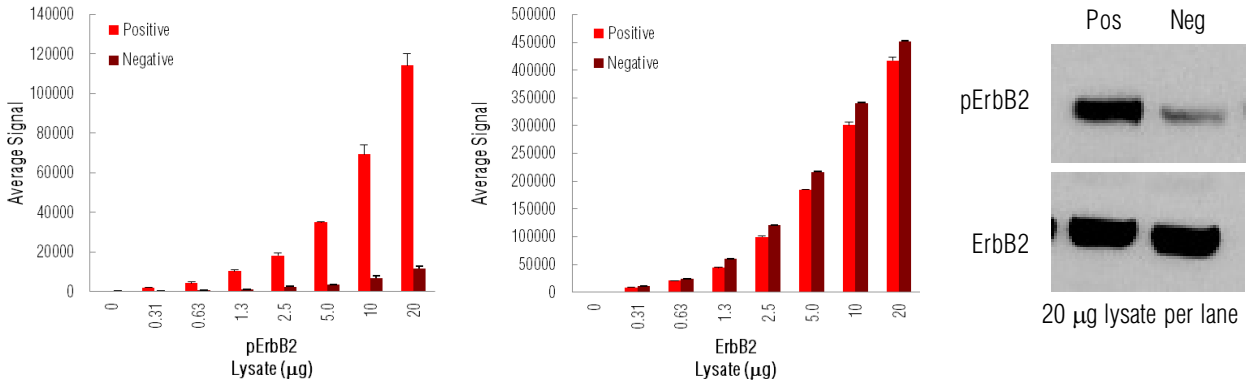


Fig. 1: Sample data generated with MULTI-SPOT Phospho(Tyr1248)/Total ErbB2 Assay. Increased signal for phosphorylated ErbB2 was observed with pErbB2 positive cell lysate. Total ErbB2 signal increased throughout the titration of both pErbB2 positive and negative cell lysates. The Phospho(Tyr1248)/Total ErbB2 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 SK-OV3 cell lysates using the MULTI-SPOT Phospho(Tyr1248)/Total ErbB2 Assay are presented below.

	Lysate (µg)	Positive			Negative			P/N
		Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	
pErbB2	0	86	13	15.1	50	21	42.0	
	0.31	2106	177	8.4	453	15	3.3	4.6
	0.63	4325	420	9.7	699	87	12.4	6.2
	1.3	10257	788	7.7	1132	97	8.6	9.1
	2.5	18123	1404	7.7	2472	432	17.5	7.3
	5.0	34926	434	1.2	3465	39	1.1	10
	10	69222	4781	6.9	6945	1071	15.4	10
	20	114128	5981	5.2	11531	1180	10.2	9.9
ErbB2	0	143	46	32.2	103	16	15.5	
	0.31	8778	370	4.2	11393	412	3.6	0.8
	0.63	20624	730	3.5	24101	3479	14.4	0.9
	1.3	44007	5903	13.4	60051	596	1.0	0.7
	2.5	99453	8015	8.1	120799	5893	4.9	0.8
	5.0	184498	7473	4.1	216863	4572	2.1	0.9
	10	300929	7352	2.4	340928	8338	2.4	0.9
	20	417262	10865	2.6	451655	10079	2.2	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. Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung KM, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. Cancer Res. 2008 Apr 15;68(8):2850-60.
2. 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.