# MSD® Phospho-ErbB2 (Tyr1248) 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-ErbB2 (Tyr1248) Whole Cell Lysate Kit					
Kit size					
1 plate	K151CLD-1				
5 plates	K151CLD-2				
20 plates	K151CLD-3				

#### Ordering information

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

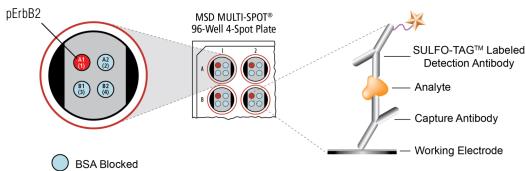
#### Company Address

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www.mesoscale.com®

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Not for use in diagnostic

procedures.

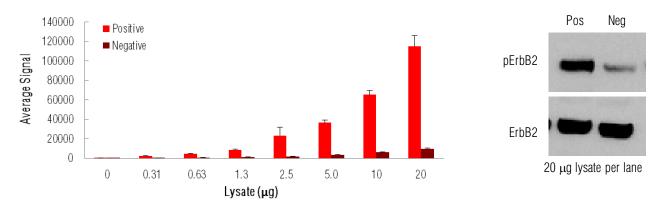


**ErbB2** (HER-2/Neu) is a 185 kDa member belonging to a family of four type I receptor-tyrosine kinases structurally consisting of an ectodomain, a single transmembrane segment, and a cytoplasmic region including a protein tyrosine kinase domain. ErbB2 currently does not have any known direct ligand, 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-ErbB2 (Tyr1248) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

#### Typical Data

Representative results for the Phospho-ErbB2 (Tyr1248) 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  $\mu$ M; 2.5 hours) (negative). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-ErbB2 antibody on one of the four spatially distinct electrodes per well. Phosphorylated ErbB2 was detected with anti-total ErbB2 antibody conjugated with MSD SULFO-TAGTM reagent.



**Fig. 1:** Sample data generated with MULTI-ARRAY® Phospho-ErbB2 (Tyr1248) Assay. Increased signal is observed with the titration of pErbB2 positive cell lysate. The Phospho-ErbB2 (Tyr1248) Assay provides a quantitative measure of the data obtained with the traditional Western blot.





## MSD Phosphoprotein Assays

#### Lysate Titration

Data for positive and negative SK-OV3 cell lysates using the MULTI-ARRAY Phospho-ErbB2 (Tyr1248) Assay are presented below.

Lysate	Positive			Negative			D/N
(µg)	Average Signal	StdDev	%CV	Average Signal	StdDev	%CV	P/N
0	133	61	45.9	93	31	33.3	
0.31	2173	467	21.5	501	78	15.6	4.3
0.63	4545	519	11.4	777	42	5.4	5.8
1.3	8606	564	6.6	1208	127	10.5	7.1
2.5	23246	8433	36.3	2046	113	5.5	11
5.0	36696	2667	7.3	3523	189	5.4	10
10	65322	4131	6.3	6051	757	12.5	11
20	114708	11285	9.8	9713	598	6.2	12

### MSD Advantage

- **Multiplexing:** Multiple analytes can be measured in one well using typical sample amounts of 25  $\mu$ g/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. 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.

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