

# MSD® Phospho-VEGFR-2 (Tyr1054) 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-VEGFR-2 (Tyr1054) Whole Cell Lysate Kit |           |
| Kit size                                        |           |
| 1 plate                                         | K151DJD-1 |
| 5 plates                                        | K151DJD-2 |
| 20 plates                                       | K151DJD-3 |

|                                       |         |
|---------------------------------------|---------|
| Phospho-VEGFR-2 Whole Cell Lysate Set |         |
| 200 µg                                | C11CI-1 |

## Ordering information

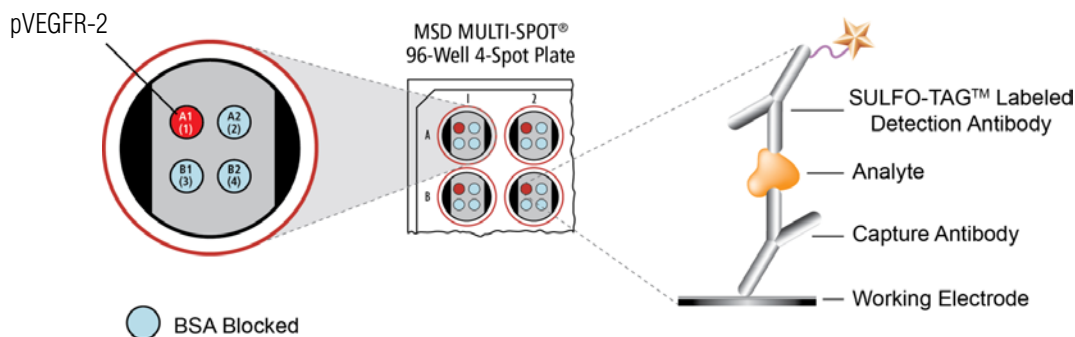
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**For Research Use Only.**  
**Not for use in diagnostic procedures.**

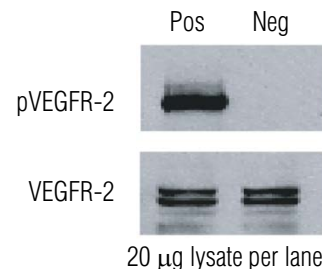
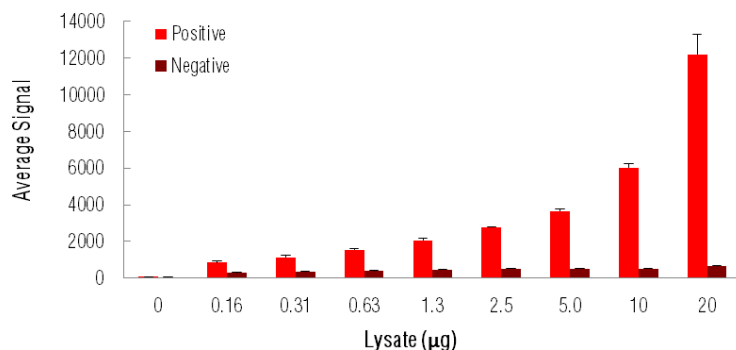


**Vascular endothelial growth factor receptor-2 (VEGFR-2)**, also known as KDR (human) and Flk1 (mouse), is an endothelial cell receptor tyrosine kinase with seven extracellular Ig-like domains. This receptor contains a ligand binding site, a single transmembrane domain, and an intracellular region containing the tyrosine kinase domain split by a non-catalytic kinase insert domain. Upon binding its ligand, VEGF, VEGFR-2 undergoes dimerization that in turn activates its tyrosine kinase activity, resulting in the auto-phosphorylation of several tyrosines (Tyr951/996 and Tyr1054/1059). Phosphorylated VEGFR-2 interacts with adaptor proteins and the integrin receptor  $\alpha V\beta 3$  to activate the SAPK2/p38 pathway. Activated VEGFR-2 also facilitates the formation of focal adhesions through HSP90-activation of FAK. Ligand-independent activation of VEGFR-2 and the subsequent activation of PI-3K/ Akt and eNOS has been observed with fluid shear stress in blood vessels, thereby leading to the production of NO and regulation of vascular homeostasis. VEGFR-2 is a critical mediator of the angiogenic functions of VEGF, and is an attractive target for anti-angiogenic drugs relating to disease states such as cancer, macular degeneration, and diabetic retinopathy.

The MSD Phospho-VEGFR-2 (Tyr1054) Assay is available on 96-well 4-Spot plates. This datasheet outlines the performance of the assay.

## Typical Data

Representative results for the Phospho-VEGFR-2 (Tyr1054) Assay are illustrated below. The signal and ratio values provided below are example data; individual results may vary depending upon the samples tested. Logarithmically growing HEK-KDR cells expressing VEGFR-2 (negative) were treated with VEGF (5 minutes; 1 nM) (positive). Whole cell lysates were added to MSD MULTI-SPOT® 4-Spot plates coated with anti-phospho-VEGFR-2 antibody on one of the four spatially distinct electrodes per well. Phosphorylated VEGFR-2 was detected with anti-total VEGFR-2 antibody conjugated with MSD SULFO-TAG™ reagent. Western blot analyses of each lysate type were performed with phospho-VEGFR-2 (Tyr1054) and total VEGFR-2 antibodies and are shown below for comparison.



**Fig. 1:** Sample data generated with MULTI-ARRAY® Phospho-VEGFR-2 (Tyr1054) Assay. Increased signal is observed with the titration of pVEGFR-2 positive cell lysate. Signal for negative lysate remains low throughout the titration. The Phospho-VEGFR-2 (Tyr1054) Assay provides a quantitative measure of the data obtained with the traditional Western blot.

# MSD Phosphoprotein Assays

## Lysate Titration

Data for positive and negative HEK-KDR cell lysates using the MULTI-ARRAY Phospho-VEGFR-2 (Tyr1054) Assay are presented below.

| Lysate (µg) | Positive       |        |      | Negative       |        |     | P/N |
|-------------|----------------|--------|------|----------------|--------|-----|-----|
|             | Average Signal | StdDev | %CV  | Average Signal | StdDev | %CV |     |
| 0           | 70             | 11     | 15.7 | 64             | 4      | 6.3 |     |
| 0.16        | 848            | 76     | 9.0  | 295            | 22     | 7.5 | 2.9 |
| 0.31        | 1148           | 84     | 7.3  | 349            | 18     | 5.2 | 3.3 |
| 0.63        | 1555           | 70     | 4.5  | 393            | 22     | 5.6 | 4.0 |
| 1.3         | 2053           | 149    | 7.3  | 467            | 32     | 6.9 | 4.4 |
| 2.5         | 2773           | 26     | 0.9  | 508            | 26     | 5.1 | 5.5 |
| 5.0         | 3665           | 101    | 2.8  | 526            | 22     | 4.2 | 7.0 |
| 10          | 6018           | 239    | 4.0  | 522            | 13     | 2.5 | 12  |
| 20          | 12207          | 1065   | 8.7  | 647            | 30     | 4.6 | 19  |

## 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](http://www.mesoscale.com)

## References using MSD's technology for the measurement of phosphoproteins

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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.
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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.

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