



Product Information

Anti-phospho-VEGF R2 [pTyr¹⁰⁵⁴]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **V 5389**

Product Description

Anti-phospho-VEGF R2 [pTyr¹⁰⁵⁴] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human VEGF R2 that contains tyrosine 1054 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide.

The antibody detects mouse VEGF R2. Human and rat (100% homologous) VEGFR2 have not been tested, but are expected to react. VEGFR3 (100%) has not been tested, but is expected to react. It has been used in immunoblotting applications.

Vascular endothelial growth factor receptor 2 (VEGFR2, also known as KDR/FLK-1) is a 200 kDa member of a receptor tyrosine kinase family whose activation plays an essential role in a large number of biological processes such as embryonic development, wound healing, cell proliferation, migration and differentiation. Like other growth factor receptors upon ligand binding, VEGFR2 forms a dimer and is autophosphorylated on multiple tyrosine residues. These sites can be involved in the regulation of kinase activity or serve as binding sites for SH2- and phosphotyrosine binding containing signaling proteins.

Phosphorylation of tyrosines 1054 and 1059 in the activation loop is required for activation of VEGFR2 and its intrinsic tyrosine kinase activity.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

Store at -70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

A recommended working concentration of 0.35 to 1.0 µg/mL is determined by immunoblotting using NIH3T3 cells transfected with full-length, wild type VEGFR2, or Porcine Aortic Epithelial (PAE) cells transfected with a chimeric receptor consisting of the extracellular domain of the CSF-1 receptor coupled to the transmembrane and cytoplasmic domains of the VEGFR2.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

1. Extracts prepared from PAE cells transfected with a chimeric receptor or from NIH3T3 cells transfected with human VEG FR2 were stimulated with VEGF (1-4, 6) or left unstimulated (5), then resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:

Lane 1, 5 & 6	no peptide
Lane 2	non-phosphorylated peptide corresponding to the immunogen
Lane 3	a generic phosphotyrosine containing peptide
Lane 4	immunogen

4. After preincubation membranes were incubated with 0.50 µg/mL VEGF R2 [pTyr¹⁰⁵⁴] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')₂ anti-rabbit IgG alkaline phosphatase and signals were detected.

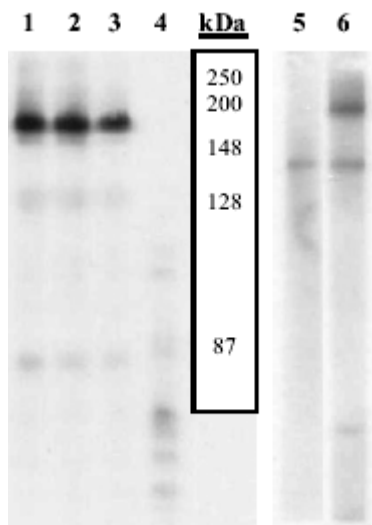


Figure 1 Peptide Competition

The data show that only the peptide corresponding to VEGF R2 [pTyr¹⁰⁵⁴] blocks the antibody signal, and the stimulation of the phospho signal after VEGF is added (compare lanes 5 and 6), demonstrating the specificity of the antibody. Note that the full-length wild type VEGF R2 is ~200 kDa (5, 6), whereas, the chimeric CSF-1-VEGF R2 is ~190 kDa (1-4). The second band detected at ~140 kDa in lanes 5 and 6 was observed in some but not all transfected NIH3T3 cell preparations and with multiple VEGF R2 antibodies.

References

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2. Sato, Y., et al., Properties of two VEGF receptors, Flt-1 and KDR, in signal transduction. *Ann. N.Y. Acad. Sci.* **902**, 201-207(2000).
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