Product name: Anti-VEGF Receptor 2 (phospho Y1054 + Y1059) antibody ab5473

Description: Rabbit polyclonal to VEGF Receptor 2 (phospho Y1054 + Y1059)

Host species: Rabbit

Tested applications: Suitable for: ICC/IF, WB

Species reactivity: Reacts with: Human

Predicted to work with: Mouse, Rat

Immunogen: Synthetic phospho peptide (Human) containing Tyrosines 1054 and 1059. This sequence is conserved in mouse and rat.

Positive control: IF: VEGF treated HUVEC whole cells. WB: VEGF treated HUVEC, MDA-MB-231 and MCF7 whole cell extract.

General notes: Reproducibility is key to advancing scientific discovery and accelerating scientists’ next breakthrough.

Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.

We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee.

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been “predicted to work with,” however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.
Form
Liquid

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.30
Preservative: 0.05% Sodium azide
Constituents: PBS, 0.1% BSA, 50% Glycerol

Purity
Immunogen affinity purified

Purification notes
The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated VEGFR 2. The final product is generated by affinity chromatography using a VEGFR 2 derived peptide that is phosphorylated at Tyrosines 1054 and 1059.

Clonality
Polyclonal

Isotype
IgG

Applications
Our Abpromise guarantee covers the use of ab5473 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>🌟🌟🌟🌟🌟</td>
<td>1/250.</td>
</tr>
<tr>
<td>WB</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use a concentration of 0.1 - 1 µg/ml. Detects a band of approximately 200 kDa.</td>
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Target

Function
Receptor for VEGF or VEGFC. Has a tyrosine-protein kinase activity. The VEGF-kinase ligand/receptor signaling system plays a key role in vascular development and regulation of vascular permeability. In case of HIV-1 infection, the interaction with extracellular viral Tat protein seems to enhance angiogenesis in Kaposi’s sarcoma lesions.

Involvement in disease
Defects in KDR are associated with susceptibility to hemangioma capillary infantile (HCI) [MIM:602089]. HCl are benign, highly proliferative lesions involving aberrant localized growth of capillary endothelium. They are the most common tumor of infancy, occurring in up to 10% of all births. Hemangiomas tend to appear shortly after birth and show rapid neonatal growth for up to 12 months characterized by endothelial hypercellularity and increased numbers of mast cells. This phase is followed by slow involution at a rate of about 10% per year and replacement by fibrofatty stroma.

Sequence similarities
Belongs to the protein kinase superfamily. Tyr protein kinase family. CSF-1/PDGFR receptor subfamily.
Contains 7 Ig-like C2-type (immunoglobulin-like) domains.
Contains 1 protein kinase domain.

Post-translational modifications
Phosphorylated. Dephosphorylated by PTPRB. Dephosphorylated by PTPRJ at Tyr-951, Tyr-996, Tyr-1054, Tyr-1059, Tyr-1175 and Tyr-1214.
Cellular localization

Membrane.

Immunofluorescence analysis of HUVEC cells labelling VEGF Receptor 2 (phospho Y1054 + Y1059) (Panel a: green) using ab5473 at 2 µg/ml in 0.1% BSA for 3 hours at room temperature, followed by Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal at 1/2000 dilution. Panel b: Nuclei stained with DAPI (blue), Panel c: F-actin stained with Alexa Fluor® 555 Rhodamine Phalloidin (red), Panel d: Merged images. The images were captured at 60X magnification.

Prior antibody incubation, HUVEC (Human umbilical vein endothelial cell line) cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes and blocked with 1% BSA for 1 hour at room temperature, followed by treatment with 100 ng of VEGF for 30 minutes. Assay was done on 90% confluent log phase HUVEC cells.

**All lanes**: Anti-VEGF Receptor 2 (phospho Y1054 + Y1059) antibody (ab5473) at 1/1000 dilution

**Lane 1**: HUVEC (Human umbilical vein endothelial cell line) whole cell extract with Skimmed milk

**Lane 2**: HUVEC cells treated with 25ng/ml VEGF for 5 minutes with Skimmed milk

**Lane 3**: MDA-MB-231 (human breast adenocarcinoma cell line) whole cell extract with Skimmed milk

**Lane 4**: MCF7 (human breast adenocarcinoma cell line) whole cell extract with Skimmed milk

Lysates/proteins at 30 µg per lane.

Blocking peptides at 5 % per lane.

**Secondary**

**All lanes**: Goat anti-rabbit IgG HRP conjugate at 1/5000 dilution

A 130 kDa band corresponding to VEGFR (pYpY1054/1059) was observed across cell lines tested.

Protein samples were electrophoresed using Novex® NuPAGE®
10 % Bis-Tris gel, XCell SureLock™ Electrophoresis System and Novex® Sharp Pre-Stained Protein Standard.

Resolved proteins were then transferred onto a nitrocellulose membrane by over night wet transfer.

Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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