Product datasheet

Anti-VEGFA antibody [EP1176Y] ab52917

Overview

Product name  Anti-VEGFA antibody [EP1176Y]
Description  Rabbit monoclonal [EP1176Y] to VEGFA
Host species  Rabbit
Specificity  The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse. This antibody fails to detect endogenous natural samples in WB.
Tested applications  Suitable for: IP, Flow Cyt, IHC-P, ICC/IF, WB
Species reactivity  Reacts with: Mouse, Human
Immunogen  Synthetic peptide within Human VEGFA aa 200-300 (C terminal). The exact sequence is proprietary.
Positive control

Purchase matching WB positive control:
Recombinant Human VEGFA protein


General notes  A trial size is available to purchase for this antibody.
Our RabMab® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents
We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
This product is a recombinant rabbit monoclonal antibody.

Properties

Form  Liquid
Storage buffer  pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, PBS, 0.05% BSA

Purity: Protein A purified
Clonality: Monoclonal
Clone number: EP1176Y
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab52917 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>IP</td>
<td>1/50</td>
<td></td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>1/30*</td>
<td>ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/100 - 1/1000 dilution.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>1/100*</td>
<td>The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse. See IHC antigen retrieval protocols.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>1/250 - 1/500</td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>1/10000. Detects a band of approximately 23 kDa (predicted molecular weight: 27 kDa). This antibody fails to detect endogenous natural samples in WB.</td>
<td></td>
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</table>

Target

Function: Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels. Binds to the FLT1/VEGFR1 and KDR/VEGFR2 receptors, heparan sulfate and heparin. NRP1/Neuropilin-1 binds isoforms VEGF-165 and VEGF-145. Isoform VEGF165B binds to KDR but does not activate downstream signaling pathways, does not activate angiogenesis and inhibits tumor growth.

Tissue specificity: Isoform VEGF189, isoform VEGF165 and isoform VEGF121 are widely expressed. Isoform VEGF206 and isoform VEGF145 are not widely expressed.

Involvement in disease: Defects in VEGFA are a cause of susceptibility to microvascular complications of diabetes type 1 (MVCD1) [MIM:603933]. These are pathological conditions that develop in numerous tissues and organs as a consequence of diabetes mellitus. They include diabetic retinopathy, diabetic nephropathy leading to end-stage renal disease, and diabetic neuropathy. Diabetic retinopathy remains the major cause of new-onset blindness among diabetic adults. It is characterized by vascular permeability and increased tissue ischemia and angiogenesis.

Sequence similarities: Belongs to the PDGF/VEGF growth factor family.
Cellular localization

Secreted. VEGF121 is acidic and freely secreted. VEGF165 is more basic, has heparin-binding properties and, although a significant proportion remains cell-associated, most is freely secreted. VEGF189 is very basic, it is cell-associated after secretion and is bound avidly by heparin and the extracellular matrix, although it may be released as a soluble form by heparin, heparinase or plasmin.

Images

All lanes: Anti-VEGFA antibody [EP1176Y] (ab52917) at 1 µg/ml (unpurified)

Lane 1: Recombinant Human VEGFA protein (ab204773)
Lane 2: Recombinant mouse VEGFA protein (Active) (ab185265)

Lysates/proteins at 0.1 µg per lane.

Secondary

All lanes: Rabbit IgG secondary antibody at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 27 kDa

Observed band size: 23 kDa

why is the actual band size different from the predicted?

Exposure time: 10 seconds

This blot was produced using a 10% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab52917 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue sections labeling VEGFA with purified ab52917 at 1:100 dilution (2.96 µg/ml).

Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0).

**Negative control** shown in inset.

Purified ab52917 staining VEGF in NIH/3T3 (Mouse embryo fibroblast cell line) cells.

The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with purified ab52917 at 5 µg/ml and ab195884, at 1/250 dilution, overnight at +4°C, followed by a further incubation at room temperature for 1h with an Goat anti-Rabbit Alexa Fluor®488 secondary (ab150081) at 2 µg/ml (shown in green).

Nuclear DNA was labeled in blue with DAPI.
Unpurified ab52917 staining VEGF in mouse kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with paraformaldehyde, permeabilized with 0.3% Triton X-100 and blocked with 5% serum for 45 minutes at 25°C. Samples were incubated with primary antibody (1/400 in 4% BSA + 5% serum in PBST) for 14 hours at 4°C. An Alexa Fluor® 546-conjugated Donkey anti-rabbit IgG polyclonal (1/300) was used as the secondary antibody.

Anti-VEGFA antibody [EP1176Y] (ab52917) at 1/10000 dilution (Purified) + His-tagged Human VEGFA (aa 27-232) recombinant protein at 0.015 µg with 5% NFDM/TBST

**Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

**Predicted band size:** 27 kDa

**Observed band size:** 27 kDa

Flow Cytometry analysis of NIH/3T3 (Mouse embryonic fibroblast) cells labeling VEGFA with purified ab52917 at 1:30 dilution (10 µg/ml) (red).

Cells were fixed with 4% paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black).

Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).
Overlay histogram showing NIH/3T3 (Mouse embryo fibroblast cell line) cells stained with unpurified ab52917 (red line).

The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab52917, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730, 0.1 μg/1x10⁶ cells used under the same conditions.

Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in NIH/3T3 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used at 1/100 dilution.

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