Anti-Factor VIII antibody [EPR24039-262] ab275376

Overview

Product name: Anti-Factor VIII antibody [EPR24039-262]
Description: Rabbit monoclonal [EPR24039-262] to Factor VIII
Host species: Rabbit
Tested applications: Suitable for: WB, IHC-P, ICC, Flow Cyt

Unsuitable for: IP
Species reactivity: Reacts with: Mouse, Rat, Human

Immunogen: Recombinant fragment within Human Factor VIII aa 1 to the C-terminus. The exact sequence is proprietary.
Database link: P00451

Positive control: WB: Human plasma; HUVEC whole cell lysate. IHC-P: Human colon carcinoma and bladder cancer tissue; Mouse spleen and lung tissue; Rat lung tissue. ICC: HUVEC cells. Flow cyt: HUVEC cells.

General notes: This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production
For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Properties

Form: Liquid
Storage buffer: Preservative: 0.01% Sodium azide
Constituents: 59.94% PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified
Clonality: Monoclonal
Clone number  EPR24039-262
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab275376 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<td>WB</td>
<td></td>
<td>1/1000. Predicted molecular weight: 267 kDa.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.</td>
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<tr>
<td>ICC</td>
<td></td>
<td>1/1000.</td>
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<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/500.</td>
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Application notes  Is unsuitable for IP.

Target

Function  Factor VIII, along with calcium and phospholipid, acts as a cofactor for factor IXa when it converts factor X to the activated form, factor Xa.

Involvement in disease  Defects in F8 are the cause of hemophilia A (HEMA) [MIM:306700]. A disorder of blood coagulation characterized by a permanent tendency to hemorrhage. About 50% of patients have severe hemophilia resulting in frequent spontaneous bleeding into joints, muscles and internal organs. Less severe forms are characterized by bleeding after trauma or surgery. Note=Of particular interest for the understanding of the function of F8 is the category of CRM (cross-reacting material) positive patients (approximately 5%) that have considerable amount of F8 in their plasma (at least 30% of normal), but the protein is non-functional; i.e., the F8 activity is much less than the plasma protein level. CRM-reduced is another category of patients in which the F8C antigen and activity are reduced to approximately the same level. Most mutations are CRM negative, and probably affect the folding and stability of the protein.


Domain  Domain F5/8 type C 2 is responsible for phospholipid-binding and essential for factor VIII activity.

Post-translational modifications  Sulfation on Tyr-1699 is essential for binding vWF.

Cellular localization  Secreted > extracellular space.

Images
Immunohistochemical analysis of paraffin-embedded Human colon carcinoma tissue labeling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on the blood vessels in human colon carcinoma (PMID: 18846440). The section was incubated with ab275376 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND™ RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HEK-293 (Human embryonic kidney epithelial cell line, Left) / HUVEC (Human umbilical vein endothelial cell, Right) cells labelling Factor VIII with ab275376 at 1/500 dilution.

A Goat anti rabbit IgG (Alexa Fluor®, ab150077) at 1/2000 dilution was used as the secondary antibody.

Negative control: HEK-293 (PMID: 31727959). HUVEC cells expressing Factor VIII is reported in literature (PMID: 30814851).

Immunofluorescent analysis of 100% methanol-fixed HUVEC cells labelling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic staining in HUVEC cell line. Negative control: HEK-293 (PMID: 31727959). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.
Western blot - Anti-Factor VIII antibody [EPR24039-262] (ab275376)

Anti-Factor VIII antibody [EPR24039-262] (ab275376) at 1/1000 dilution + Human plasma at 20 µl

**Secondary**

Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

**Predicted band size:** 267 kDa

**Observed band size:** 210-280, 330 kDa

*why is the actual band size different from the predicted?*

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID: 3085106).

Exposure time: 10 seconds.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Factor VIII antibody [EPR24039-262] (ab275376)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labeling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Positive staining on rat lung (PMID: 25911555). The section was incubated with ab275376 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.
Western blot - Anti-Factor VIII antibody [EPR24039-262] (ab275376)

All lanes: Anti-Factor VIII antibody [EPR24039-262] (ab275376)
at 1/1000 dilution

Lane 1: HUVEC (human umbilical vein endothelial cell), whole cell lysate
Lane 2: HEK-293 (human embryonic kidney epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 267 kDa
Observed band size: 166,210-280,330 kDa why is the actual band size different from the predicted?

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Negative control: HEK-293 (PMID: 27208572).
The molecular weight observed is consistent with what has been described in the literature (PMID: 3085106).

Exposure time: 26 seconds.
Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labeling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Positive staining on mouse lung (PMID: 25911555). The section was incubated with ab275376 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Immunohistochemical analysis of paraffin-embedded Mouse spleen tissue labeling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Positive staining on the megakaryocytes and platelets in mouse spleen (PMID: 25911555). The section was incubated with ab275376 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.
Immunohistochemical analysis of paraffin-embedded Human bladder cancer tissue labeling Factor VIII with ab275376 at 1/1000 (0.457 ug/ml) dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on the blood vessels in human bladder cancer. The section was incubated with ab275376 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

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