monoclonal antibody anti-human human factor VIII IgG
REF ESH-8

Description
For Research Use Only

Factor VIII (fVIII) is a glycoprotein essential for the intrinsic pathway of blood coagulation because of its ability to accelerate the proteolytic activation of Factor X (fIX) by the serine protease Factor IXa (fIXa). Synthesized mainly in hepatocytes, the mature form of fVIII is a single-chain, 2332 amino acid polypeptide, with a molecular ratio of approximately 265,000 Daltons. The molecule is comprised of two homologous groups separated by a third segment and organized with the domain structure of A1-A2-B-A3-C1-C2. Cleaved intracellularly into a two-chain heterodimer, a heavy-chain of domains A1-A2-B and a light-chain of domains A3-C1-C2, fVIII is secreted into the bloodstream and forms a stable, non-covalent complex with von Willebrand Factor (vWF). fVIII is activated by proteolytic cleavage and released from its vWF carrier protein by thrombin. Factor VIIIa consists of the domains A1-A2 and the A3-C1-C2 light chain, both of which are necessary for sustained activity. The B domain does not contribute to the active molecule and is lost after activation. fVIIIa is a cofactor for fIXa along with calcium and phospholipids. Binding to phospholipids and to platelets occurs via the light chain and has been determined to be associated with sequences within the C domain. The light chain is also responsible for the binding to vWF.

REF ESH-8 is a monoclonal antibody directed against human factor VIII, reactive with an epitope in the C2 domain of the light chain, amino acids 2248-2285. The antibody shows no reactivity with human von Willebrand Factor antigen (vWF:Ag).

Preparation
REF 3936 is a murine IgG<sub>2a</sub> monoclonal antibody is purified from cell culture via Protein G affinity chromatography. Purified human factor VIII:C cryoprecipitate was used as the immunizing antigen. Hybridomas were created by fusion of the immunized spleen cells with NS-1 murine myeloma cell lines.

Species Cross-Reactivity
REF ESH-8 has been found to react with factor VIII of various species via IRMA, having set its reactivity with factor VIII from human plasma as 100%.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reactivity (%)</th>
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<tbody>
<tr>
<td>Baboon</td>
<td>118%</td>
</tr>
<tr>
<td>Canine</td>
<td>56%</td>
</tr>
<tr>
<td>Feline</td>
<td>58%</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>69%</td>
</tr>
<tr>
<td>Porcine</td>
<td>NA</td>
</tr>
<tr>
<td>Rabbit</td>
<td>NA</td>
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Clot Inhibition
Following incubation for 2 hours at 37°C, REF ESH-8 at 1 µg/mL demonstrated a maximum inhibition of 72%.

Applications
A. Immunopurification/Immunodepletion
REF ESH-8 binds factor VIII:C from plasma when covalently coupled to gels (Reference 1).

B. Immunohistochemistry
REF ESH-8 has successfully stained formalin fixed-paraffin embedded cardiac atherosclerotic tissue (Reference 7).

C. Western blot
REF ESH-8 is not conformation dependent and is useful for Western blotting if a chemiluminescent detection system, e.g. Amersham ECL is used (peroxidase detection systems do not have sufficient sensitivity). Positive results may not be assured due to the sample stability, amount of sample applied and the characteristics of the antibody.

References

Related Products
Human vWF FVIII-free, REF 2042. IMUBIND<sup>®</sup> vWF ELISA, REF 828. IMUBIND<sup>®</sup> vWF Activity ELISA, REF 885. IMUBIND<sup>®</sup> FVIII ELISA, REF 884CON.