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Monoclonal antibody against human Factor VIII

Product No. ADGESH-8

Description

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 (FX) by the serine protease Factor IXa (FIXa). Synthesized mainly in hepatocytes, the mature form of FVIII is a single-chain protein with a molecular ratio of 265 kDa. 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 blood stream 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.

Properties

The antibody (clone ESH-8, subclass IgG_{2a}) is purified from cell culture via Protein G affinity chromatography. Purified human factor VIII:C cryoprecipitate was used as the immunizing antigen.
The antibody is 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).
The cross-reactivity was tested via IRMA, in comparison with human plasma (100%):

Baboon	Feline	Guinea pig	Porcine
118 %	56%	58%	69%

Presentation

Screw capped vial containing 0.5 mg of purified antibody in PBS, pH 7.2, sterile. The IgG concentration is 1 mg/ml. Spin the vial briefly before opening.

Storage and Stability

Store the antibody at 2°-8°C. For long-term storage the antibody should be aliquoted and stored at –20°C or colder. It is recommended to avoid freeze-thaw cycles.

Applications

A. Immunopurification / Immunodepletion

The antibody binds factor VIII:C from plasma when covalently coupled to gels [(1)].

B. Immunohistochemistry

The antibody has successfully stained formalin fixed-paraffin embedded cardiac atherosclerotic tissue. [(7)].

C. Inhibitor

Clot inhibition was tested by incubation for 2 hrs at 37°C. 72% inhibition at 1 µg/mL (highest conc. tested) [(6)].

D. Western Blot

The antibody 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

1. The production and characterization of a panel of ten murine monoclonal antibodies to human procoagulant factor VIII. Griffin BD *et al*. *Thromb Haemost*, 1986; 55: 40-46.
2. Development, optimization and use of an enzyme linked immunosorbent assay (ELISA) to measure factor VIII antigen utilizing monoclonal antibodies. Hornsey VS *et al. Transf Med*, 1992; 2: 223-229.
3. In vivo production of human factor VII in mice after intrasplenic implantation of primary fibroblasts transfected by receptor-mediated, adenovirus-augmented gene delivery. Zatloukal K *et al. Proc Nat Acad Science USA,* 1994; 91: 5148-5152.
4. Some factor VIII inhibitor antibodies recognize a common epitope corresponding to C2 domain amino acids 2248 through 2312, which overlap a phospholipid-binding site. Scandella D *et al. Blood*, 1995; 86: 1811-1819.
5. Slowed release of thrombin-cleaved factor VIII from von Willebrand factor by a monoclonal and a human antibody is a novel mechanism for factor VIII inhibition. Saenko EL et al. J Biol Chem. 1996 Nov 1;271(44):27424-27431.
6. Low molecular weight peptides restore the procoagulant activity of factor VIII in the presence of the potent inhibitor antibody ESH8. Villard S et al. Journal of Biological Chemistry 2002, 277: 27232-27239.
7. Intrinsic pathway of blood coagulation contributes to thrombogenicity of atherosclerotic plaque. Ananyeva NM et al. Blood 2002, 99: 4475-4485.

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