

monoclonal antibody against human Factor VIII

REF ESH-4

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 (fX) 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 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.

ESH-4 is a monoclonal antibody directed against human factor VIII, reactive with an epitope in the C2 domain of the light chain, amino acids 2303-2332. The antibody shows no reactivity with human von Willebrand Factor antigen (vWF:Ag).

Preparation

ESH-4 is a murine IgG_{2a} monoclonal antibody 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.

Presentation

Screw-capped glass vial containing 0.5 mg of purified IgG lyophilized from a 0.5 mL solution of 0.15M Phosphate Buffered Saline, 100 mM Mannitol, pH 7.4.

Reconstitution

Add 0.5 mL of filtered deionized or sterile water to generate a 1.0 mg/mL stock solution.

Storage

Store lyophilized antibody at 2°-8°C. Aliquot and store reconstituted antibody at -20°C or colder.

Species Cross-Reactivity

ESH-4 has been found to react with baboon and rabbit factor VIII via IRMA, having set its reactivity with factor VIII from human plasma as 100%. Reactivity with canine, feline, guinea pig (rodent) and porcine

Baboon	Canine	Feline	Guinea Pig	Porcine	Rabbit
112%	NA	NA	NA	NA	145%

Clot Inhibition

Following incubation for 2 hours at 37°C, ESH-4 at a concentration of 3 µg/mL demonstrated an inhibition of 89%. ESH-4 was shown to inhibit FVIII binding to phosphatidylserine (PS) (Reference 6).

Applications

A. Immunopurification/Immunodepletion

ESH-4 binds factor VIII:C from plasma when covalently coupled to chromatography gels (Reference 1).

B. Immunofluorescence Microscopy

ESH-4 is useful for immunofluorescence (Reference 3).

C. Western blot

ESH-4 is a conformation dependent antibody and is **not** useful for Western blotting.

References

1. Griffin, B. D., *et al.* *Thrombosis and Haemostasis* 1986, **55**: 40-46.
2. Hornsey, V. S., *et al.* *Transfusion Medicine* 1992, **2**: 223-229.
3. Zatloukal, K., *et al.* *Proceeds of the National Academy of Science USA* 1994, **91**: 5148-5152.
4. Scandella, D., *et al.* *Blood* 1995, **86**: 1811-1819.
5. Seanko, E. L., *et al.* *Journal of Biological Chemistry* 1997, **272**: 18007-18014.
6. Lewis, D. A., *et al.* *Thrombosis and Haemostasis* 2003, **89**: 795-802.
6. Wakabayashi, H. *et al.* *Journal of Biological Chemistry* 2013, **288**(43): 31289-31297.

Related Products

vWF, human (FVIII-free), REF 2042
IMUBIND® vWF ELISA, REF 828
IMUBIND® vWF Activity ELISA, REF 885

Warnings and Precautions

This product contains source material of a biological origin. Use Universal Precautions.

Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Temperature Limitation
	Lot Number
	Expiration Date
	Catalog Number