



Murine Anti-Factor VIII

Clone 012

Factor VIII (FVIII) is a heterodimer consisting of a heavy chain (ranging in mass from 90 to 200 kDa) bound via metal ions to a light chain (80 kDa). In plasma, factor VIII circulates in an inactive form bound to von Willebrand factor. Following activation by factor Xa or thrombin, factor VIIIa can function as cofactor for the enzyme factor IXa in the activation of factor X in the presence of phospholipid and Ca^{2+} . Absent or defective FVIII is the cause of the X-linked recessive bleeding disorder hemophilia A. Mab HFVIII (also known as R8B12) recognizes the discontinuous epitope of residues 497-510 and 584-593 in the A2 domain of FVIII,¹ and is suitable for purification of FVIII,² ELISA, sandwich ELISA³, Western blotting, and bio-layer interferometry applications.

Description

Antibody Source:	mouse monoclonal, IgG ₁
Antigen Species Bound:	human, rhesus
Specificity:	FVIII A2 domain, (residues 497-510, 584-593) ¹
Immunogen:	human FVIII heavy chain ⁴

Formulation and Storage

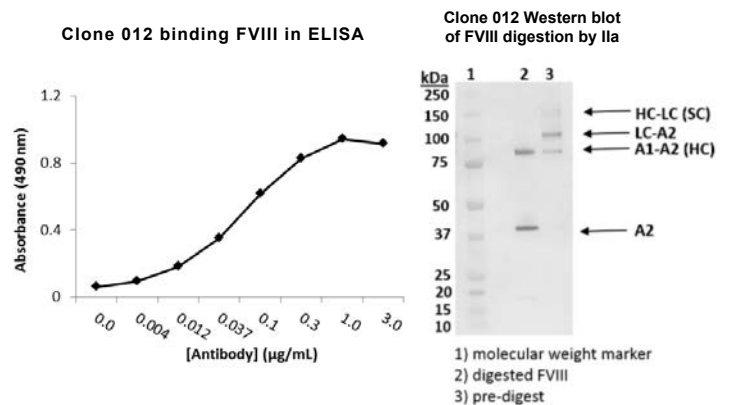
Purity:	Purified by protein G affinity chromatography from serum-free cell culture supernatant.
Product Formulation:	Lyophilized from a ≥ 1 mg/ml solution in 20 mM NaH_2PO_4 0.15 M NaCl, 1.0% (w/v) mannitol, pH 7.4. Concentration determined by absorbance measurement at 280 nm and using an extinction coefficient of 1.4 ($\epsilon_{0.1\%}$).
Reconstitution:	Reconstitute with deionized water.
Storage:	Store lyophilized or reconstituted and aliquoted material at -20°C for prolonged periods. Avoid freeze-thaw cycles. Alternatively, add 0.02% (w/v) sodium azide to reconstituted solution and store at 4°C .

Country of origin: USA

Size Options: 0.1 mg or 0.5 mg

Applications

Working Concentration:	Approximately 1-5 $\mu\text{g/ml}$. Researcher should titer antibody in specific assay.
ELISA:	Binds immobilized human and rhesus FVIII.
Immunoblotting:	Binds A2 domain of human FVIII under reduced conditions.
Inhibition:	Not inhibitory in Bethesda assay. ⁵
Affinity Constant (apparent K_D):	$K_D = 2$ nM, ($k_{\text{dis}} = 7 \times 10^{-4} \text{ sec}^{-1}$) by bio-layer interferometry.



References

- [1] C. Ansong, S.M. Miles, P.J. Fay. Epitope mapping factor VIII A2 domain by affinity-directed mass spectrometry: residues 497-510 and 584-593 comprise a discontinuous epitope for the monoclonal antibody R8B12. (2006). *J Thromb Haemost.* 4(4):842-847.
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- [3] H. Wakabayashi, A.E. Griffiths, P.J. Fay. Factor VIII Lacking the C2 Domain Retains Cofactor Activity in Vitro. (2010). *J Biol Chem.* 285(33):25176-25184
- [4] P.J. Fay, P. J. Haidaris, T.M. Smudzin. Human factor VIIIa subunit structure. Reconstruction of factor VIIIa from the isolated A1/A3-C1-C2 dimer and A2 subunit. (1991). *J Biol Chem.* 266(14):8957-8962.
- [5] R.C. Markovitz, J.F. Healey, E.T. Parker, S.L. Meeks, P. Lollar. The diversity of the immune response to the A2 domain of human factor VIII. (2013). *Blood.* 121(14):2785-2795.