



Murine Anti-Factor VIII

Clone GMA-8023

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, FVIII 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. GMA-8023 (also known as 2G10) recognizes the A2 domain of FVIII¹ and can be used in conjunction with GMA-8024 as a sandwich ELISA pair for detection of human FVIII. It does not bind murine, rhesus or cynomolgus FVIII.

Description

Antibody Source:	mouse monoclonal, IgG _{2a}
Antigen Species Bound:	human (no murine, rhesus or cynomolgus monkey binding)
Specificity:	FVIII A2 domain
Immunogen:	B-domain deleted recombinant human FVIII

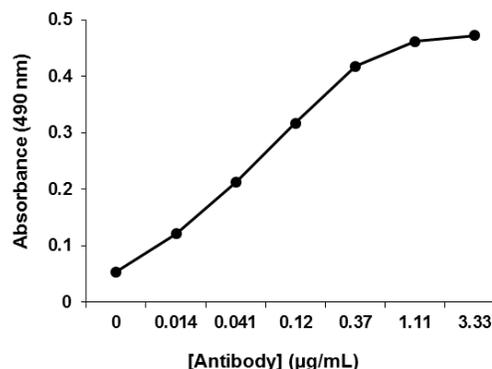
Formulation and Storage

Purity:	IgG 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:	Aliquot and store at -20°C for prolonged periods. Avoid freeze-thaw cycles. Alternatively, add 0.02% (w/v) sodium azide 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 human FVIII immobilized or in plasma.
Immunoblotting:	Does not blot.
Inhibition:	Inhibitory by aPTT clotting assay. ¹
Dissociation constant:	$k_{\text{dis}} = 2 \times 10^{-1} \text{ sec}^{-1}$ by bio-layer interferometry.

GMA-8023 binding in ELISA



References

- [1] 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.
- [2] H. Wakabayashi, M.E. Koszelak, M. Mastro, P.J. Fay. Metal ion-independent association of factor VIII subunits and the roles of calcium and copper ions for cofactor activity and inter-subunit affinity. (2001). *Biochemistry*. 40(34):10293-10300.
- [3] H. Chen, M. Shi, A. Gilam, Q. Zheng, Y. Zhang, I. Afrikanova, J. Li, Z. Gluzman, R. Jiang, L. Kong, R.Chen-Tsai. Hemophilia A ameliorated in mice by CRISPR-based in vivo genome editing of human Factor VI. (2019). *Scientific Reports*. 9:16838.
- [4] L. Zhang, B. Handyside, R. Murphy, C-R. Sihm, L. Xie, C. Vitelli, D. Harmon, S. Sisó, S. Liu, S. Bullens, S. Bunting, S. Fong. Prednisolone Does Not Regulate Factor VIII Expression in Mice Receiving AAV5-hFVIII-SQ: Valoctocogene Roxaparovec. *Molecular Therapy: Methods & Clinical Development*. (2020). 17:13-20.