



# Murine Anti-Factor IX

## Clone 001

Factor IX (FIX) is a vitamin K-dependent zymogen that plays an essential role in the coagulation cascade leading to thrombus formation. In the presence of calcium, activated Factor IX (FIXa) complexes with Factor VIIIa on phospholipid surfaces to create the tenase complex, which converts Factor X to its activated form. Absent or defective FIX is the cause of the X-linked recessive bleeding disorder hemophilia B. Mab HFIX-001 (BC2) binds to the N-terminal Gla domain of FIX and the light chain of FIXa, as detected by Western blot and ELISA. In addition, Mab HFIX-001 prolongs clotting time in aPTT-based plasma clotting assays, and prevents arterial thrombosis *in vivo* in a rat model<sup>1</sup>.

### Description

**Antibody Source:** mouse monoclonal, IgG<sub>2a</sub>

**Antigen Species Bound:** human, rat

**Specificity:** Gla domain (residues 1-40) of FIX/FIXa

**Immunogen:** human FIX

### Formulation and Storage

**Purity:** Purified by protein G affinity chromatography from serum-free cell culture supernatant.

**Product Formulation:** Lyophilized from a  $\geq 1$  mg/ml solution in 20 mM NaH<sub>2</sub>PO<sub>4</sub> 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

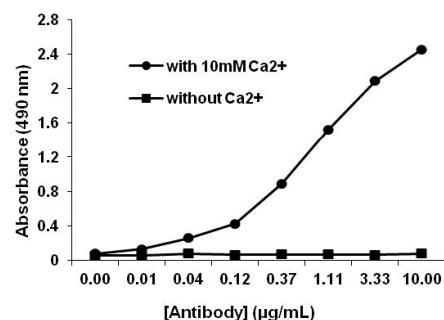
**Working Concentration:** Approximately 1-5  $\mu$ g/ml. Researcher should titer antibody in specific assay.

**ELISA:** Binds immobilized human and rat FIX and human FIXa only in the presence of calcium.

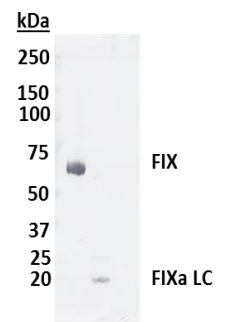
**Immunoblotting:** Western blot detects human FIX and light chain of human FIXa under reduced conditions in presence of calcium.

**Inhibition:** Prolongs plasma clot time in aPTT clotting assay.

Clone 001 binding to human FIX in ELISA



Western blot with 10 mM Ca<sup>2+</sup>



### References

- [1] G.Z. Feuerstein, J.R. Toomey, R. Valocik, P. Koster, A. Patel, M.N. Blackburn. An inhibitory anti-factor IX antibody effectively reduces thrombus formation in a rat model of venous thrombosis. (1999). *Thromb. Haemost.* 82(5):1443-1445.
- [2] G.Z. Feuerstein, A. Patel, J.R. Toomey, P. Bugelski, A.J. Nichols, W.R. Church, R. Valocik, P. Koster, A. Baker, M.N. Blackburn. Antithrombotic efficacy of a novel murine antihuman factor IX antibody in rats. (1999). *Arterioscler Thromb Vasc Biol.* 19(10):2554-2562.
- [3] J. Tie, D. Jin, D.L. Straight, D.W. Stafford. Functional study of the vitamin K cycle in mammalian cells. (2011). *Blood.* 117(10):2967-2974.
- [4] J.A. Haque, M.G. McDonald, J.D. Kulman, A.E. Rettie. A cellular system for quantitation of vitamin K cycle activity: structure-activity effects on vitamin K antagonism by warfarin metabolites. (2014). *Blood.* 123(4):582-589.
- [5] M. A. Chiasson, N. J. Rollins, J. J. Stephany, K. A. Sitko, K. A. Matreyek, M. Verby, S. Sun, F. P. Roth, D. DeSloover, D. S. Marks, A. E. Rettie, D. M. Fowler. Multiplexed measurement of variant abundance and activity reveals VKOR topology, active site and human variant impact. *eLife.* (2020) 9:1-25.