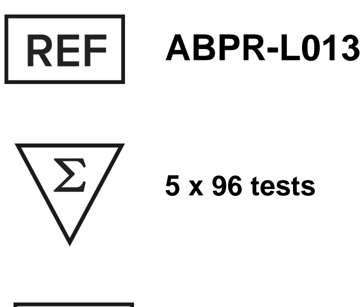
Version 19-05/24

User's Manual

Murine Factor X Matched Antibody Pair



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Murine Factor X

General Description

Factor X (F.X, Stuart Factor) is a vitamin K-dependent glycoprotein produced in the liver. The concentration of F.X in plasma is ~10 µg/ml (~170 nM). Factor X is expressed as a two-chain molecule with a molecular weight of 59 kDa. The light chain (17 kDa) of F.X contains a calcium-binding domain consisting of one hydroxyaspartic acid and 11 γ-carboxyglutamic acid (gla) residues. These residues allow F.X to bind to membranes that contain acidic phospholipids in a calcium dependent manner. This is followed by two EGF-like domains. The heavy chain of F.X (42 kDa) consists of the catalytic domain, carbohydrate and the activation peptide. Activation of F.X to the active enzyme (F.Xa) results from cleavage at residue Arg52 in the heavy chain of F.X by a complex of F.IXa, cofactor VIIIa, calcium and negatively charged phospholipid surface (the tenase complex), or by the F.VIIa-tissue factor complex. Both activation pathways result in the release of the activation peptide from the N-terminal of the heavy chain. The F.Xa generated is a serine protease responsible for the activation of prothrombin to thrombin in the presence of a phospholipid membrane, calcium and cofactor Va. The activity of F.Xa in plasma is inhibited by antithrombin (ATIII), α1 Antitrypsin, α2 macroglobulin and tissue factor pathway inhibitor (TFPI). The inhibitory activity of ATIII is stimulated approximately 1000-fold by heparin.

Principles of Testing

Factor X (FX, Stuart Factor) is a vitamin K-dependent glycoprotein produced in the liver. In human plasma the concentration of FX is ~10 µg/ml (~170 nM). Factor X is expressed as a two-chain molecule with a molecular weight of 59 kDa. The light chain (17 kDa) of FX contains a calcium-binding domain consisting of one hydroxy-aspartic acid and 11 γ-carboxyglutamic acid (gla) residues. These residues allow FX to bind to membranes that contain acidic phospholipids in a calcium dependent manner. This is followed by two EGF-like domains. The heavy chain of FX (42 kDa) consists of the catalytic domain, carbohydrate and the activation peptide. Activation of FX to the active enzyme (FXa) results from cleavage at residue Arg52 in the heavy chain of FX by a complex of F.IXa, cofactor VIIIa, calcium and negatively charged phospholipid surface (the tenase complex), or by the F.VIIa-tissue factor complex. Both activation pathways result in the release of the activation peptide from the N-terminal of the heavy chain. The FXa generated is a serine protease responsible for the activation of prothrombin to thrombin in the presence of a phospholipid membrane, calcium and cofactor Va. The activity of FXa in plasma is inhibited by antithrombin (ATIII), α1antitrypsin, α2 macroglobulin and tissue factor pathway inhibitor (TFPI). The inhibitory activity of ATIII is stimulated approximately 1000-fold by heparin.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.4 ml of affinity-purified polyclonal anti-FX antibody for coating plates.
2. Detecting Antibody (red): 0.4 ml of peroxidase conjugated polyclonal anti-FX antibody for detection of

captured murine FX.

Note: Reagents are sufficient for at least 4×96 well plates using recommended protocols. Antibodies are supplied in a 50% (v/v) glycerol solution for storage at –10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

Materials Required But Not Supplied

1. Coating Buffer: 50 mM Carbonate
2. PBS:(base for wash buffer and blocking buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Blocking Buffer: PBS-BSA(1%, w/v)
5. Sample Diluent: HBS-BSA-T20
6. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
7. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
8. Stopping Solution: 2.5 M H2SO4
9. Other: Microplates; Microplate washer; Microplate reader.

Storage

-10 to -20°C

Assay Procedure

1. **Coating of plates:** Dilute the capture antibody 1/100 in coating buffer(preferably in a polypropylene tube) and immediately add 100 µl to every well in the plate. Incubate overnight at 2-8°C.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 minutes at 22°C. Wash plate 3 times with wash buffer.
3. **Samples:** Normal mouse plasma is diluted 1/50(100%) then serial 1/2's down to 1/1600(3.13%). Sample plasmas are diluted 1/100, 1/200 and 1/400. All dilutions are made in HBS-BSA T20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
4. **Detecting Antibody:** Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample diluent and apply 100 µl to each well. Incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
5. **OPD Substrate:** Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 10-15 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at a wavelength of 490 nm.