Creative Diagnostics®

Version 17-04/25

User's Manual

Human Factor X Matched Antibody Pair

REF **ABPR-L012**

Σ **5 x 96 tests**

RUO

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human 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

Affinity-purified polyclonal antibody to FX is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing FX are applied. The coated antibody will capture the FX in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to FX is added to the plate to bind to the captured FX. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of H_2SO_4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of FX in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of affinity-purified polyclonal anti-FX antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated polyclonal anti-FX antibody for detection of captured FX.

Note: Reagents are sufficient for at least 5×96 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:** Reference plasma is diluted 1/100(100%) then serial 1/2's down to 1/3200(3.13%). Sample plasmas are diluted 1/200, 1/400 and 1/800. All dilutions are made in HBS-BSA T20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 90 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 90 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.