Version 16-04/25

User's Manual

Human Factor XI Matched Antibody Pair

REF **ABPR-L014**

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 XI

General Description

Factor XI (F.XI, plasma thromboplastin antecedent) is a coagulation protein produced in the liver that circulates in plasma at approximately 5 µg/mL (30 nM). The mass of F.XI is 160 kDa as determined by SDS-PAGE under non-reducing conditions and 80 kDa upon reduction. F.XI consists of two identical 80 kDa subunits linked by disulphide bonds. Each subunit consists of a tandem repeat of four apple domains followed by a serine protease catalytic domain. Cleavage of F.XI by activated factor XII or thrombin converts each subunit into a two-chain form and generates two active sites per F.XIa molecule. The mass of F.XIa is 160 kDa unreduced, but upon reduction F.XIa migrates as a heavy chain of 50 kDa and a light chain of 30 kDa. The catalytic site of F.XIa resides in the light chain. In plasma, F.XI or F.XIa circulates in non-covalent 1:1 complex with high molecular weight kininogen, which acts as a cofactor in the activation of F.XI by activated factor XII. The activity of F.XIa is regulated by platelets and by several proteinase inhibitors including, in order of decreasing importance, C1-inhibitor, α2 Antiplasmin, α1 Antitrypsin and antithrombin. Heparin has relatively little effect on the rate of inhibition of F.XIa by antithrombin. The only known natural substrate for activated F.XI (F.XIa) is factor IX (Christmas factor) and the only cofactor required for this reaction is ionized calcium.

Principles of Testing

Affinity-purified antibody to FXI 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 FXI are applied. The coated antibody will capture the FXI in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to FXI is added to the plate to bind to the captured FXI. 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 FXI present in the sample.

Reagents And Materials Provided

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

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 for 2 hours at 22°C or 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 1/50(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-BSAT20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 60 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 60 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 5-10 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.