Version 14-04/25

User's Manual

Human Factor XII (FXII) Matched Antibody Pair

REF **ABPR-L015**

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 XII

General Description

Factor XII (F.XII, Hageman factor) is a 76 kDa, single chain glycoprotein produced in the liver. In plasma, F.XII circulates as a protease zymogen at a concentration of approximately 30 µg/ml (400 nM). Upon vascular injury F.XII binds to negatively charged extravascular surfaces such as cartilage and skin, which facilitate activation of the zymogen to the active serine protease. Cleavage of F.XII by kallikrein after residue Arg353 produces the enzyme α F.XIIa, consisting of a 28 kDa light chain containing the protease domain, and a 52 kDa heavy chain containing the anionic surface-binding domain. Substrates for surface bound F.XIIa include the zymogens prekallikrein (PK) and factor XI (F.XI) as well as the procofactor high-molecular weight kininogen (HK). The activation of these substrates results in positive feedback activation of F.XII. Further cleavage of αF.XIIa by kallikrein produces the 28 kDa fragment β F.XIIa (Hageman factor fragment). βF.XIIa has reduced procoagulant activity as it lacks the anionic surface-binding domain, but is capable of fluid-phase activation of PK, factor VII and complement C1. The activity of F.XIIa in plasma is regulated predominantly by C1-Inhibitor, with relatively minor contributions by α2 Antiplasmin, α2 macroglobulin and antithrombin, even in the presence of therapeutic levels of heparin.

Principles of Testing

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

Reagents And Materials Provided

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

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 or for 2 hours at 22°C.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 90 minutes at 22°C. Wash plate 3 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/500(100%) then serial 1/2's down to 1/16000(3.13%). Sample plasmas are diluted 1/1000, 1/2000 and 1/4000. All dilutions are made in HBS-BSA-T20 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.