Version 30-03/25

User's Manual

Human Factor VII Matched Antibody Pair

REF **ABPR-L006**

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 VI

General Description

Factor VII (F.VII, also known as Stable Factor and Proconvertin) is a vitamin K-dependent glycoprotein produced in the liver. Plasma concentration of F.VII is normally ~0.5 µg/ml (10 nM) in plasma. A deficiency of F.VII is associated with bleeding in a clinical pattern similar to haemophilia, but is inherited as an autosomal recessive trait. The deficiency can be characterized by a quantitative (low activity and low antigen) or a qualitative (low activity and normal antigen) defect in F.VII function. In its zymogen form F.VII is a single chain molecule of ~50 kDa. It contains two EGF-like domains and an amino-terminal domain containing 10 γ-carboxyglutamic acid (Gla) residues. These Gla residues allow F.VII to bind divalent metal ions and participate in calcium-dependent binding interactions. F.VII and activated F.VII (F.VIIa) bind to tissue factor exposed at the site of vascular injury. F.IXa, F.Xa or F.VIIa rapidly activate tissue factor-bound F.VII to F.VIIa in the presence of calcium and phospholipid. Thrombin and F.XIIa are able to activate F.VII in the fluid phase in the absence of cofactors. The activation of the single chain zymogen F.VII occurs by proteolysis after residue Arg152, resulting in a two-chain active serine protease consisting of a 30 kDa heavy chain and an 18 kDa light chain. In complex with tissue factor, phospholipid and calcium, F.VIIa is able to activate F.X and F.IX. Free F.VIIa in plasma is remarkably stable, but the activity of F.VIIa/TF complex is regulated by Tissue Factor Pathway Inhibitor (TFPI) in the presence of F.Xa, and also by Antithrombin (ATIII) in the presence of heparin.

Principles of Testing

Affinity-purified antibody to FVII is coated onto the wells of a microtitre plate. Remaining binding sites on the plate are blocked with an excess of bovine albumin. The plates are washed and plasma or other fluids containing FVII are applied. The coated antibody will capture the FVII in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to FVII is added to the plate to bind to the captured FVII. After washing the plate to remove unbound conjugate, 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 FVII present in the sample.

Reagents And Materials Provided

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

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. Sample Diluent and Wash Buffer: PBS-Tween(0.1%, v/v)
4. Blocking Buffer: PBS-BSA(1%, w/v)
5. Conjugate 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 90 minutes at 22°C. Wash plate 3 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/10(100%) then serial 1/2's down to 1/320(3.13%). Sample plasmas are diluted 1/20, 1/40 and 1/80. All dilutions are made in PBS-Tween 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 conjugate 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 10-15 minutes, and then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at wavelength of 490 nm.