Version 01-06/20

User's Manual

Human Factor V Matched Antibody Pair

REF **ABPR-L005**

Σ **Sufficient reagent for 5 x 96 well plates**

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 V

General Description

Factor V (formerly referred to as accelerator globulin and labile factor) is a large glycoprotein (320 kDa) that is produced in the liver. The gene that encodes factor V (F.V) is located on chromosome 1. A congenital deficiency of F.V is a hemorrhagic disorder inherited as an autosomal recessive disease. The concentration of F.V in plasma is typically 10 µg/ml. F.V is a pro-cofactor that is activated through limited proteolysis by thrombin, or by activated factor X in the presence of phospholipid surface. Other physiologic activators of F.V include plasmin, neutrophil elastase and platelet calpain. The activated cofactor (F.Va) is an essential component of the prothrombin activator complex, which consists of F.Va, activated factor X, calcium and anionic phospholipid surface. The intact prothrombinase complex activates prothrombin to thrombin at a rate 300,000-fold greater than activated factor X alone. In a positive feedback loop, the thrombin generated accelerates its own generation by activating more F.V to F.Va. Thrombin also acts to down-regulate F.Va indirectly by activating Protein C, which inactivates F.Va cofactor activity.

Principles of Testing

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

Reagents And Materials Provided

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

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 4°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 1/200(100%) then serial 1/2's down to 1/6400(13%). Sample plasmas are diluted 1/400, 1/800 and 1/1600. 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 wavelength of 490 nm.