Version 16-04/25

User's Manual

Human Prothrombin Matched Antibody Pair

REF **ABPR-L004**

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 Prothrombin (F2)

General Description

Thrombin (EC3.4.21.5, α-thrombin) is the product of proteolytic activation of the zymogen prothrombin. Human thrombin is a two-chain serine protease with a mass of 37 kDa. The active site is located within the heavy chain. Thrombin has a high specificity for certain arginine bonds in protein substrates. The primary substrate is fibrinogen which thrombin converts to fibrin through the cleavage of four arginyl-glycyl peptide bonds. Thrombin is also an important activator of platelets, factor XIII, Protein C and TAFI (Plasma procarboxypeptidase B). In a positive feedback mechanism, thrombin increases the rate of its own production by activation of factors VIII and V. The rate of thrombin production is subsequently limited indirectly through the activation of Protein C by thrombin, which then inactivates the activated cofactors VIII and V. The binding of thrombin to thrombomodulin on the cell surface dramatically alters thrombin's specificity, increasing it's activity toward Protein C and TAFI, and decreasing it's activity toward fibrinogen and activating cofactors VIII and V. In plasma, thrombin activity is inhibited primarily by antithrombin and to a lesser extent heparin cofactor II. The rate of inhibition by both of these inhibitors is profoundly increased in the presence of optimal concentrations of heparin. Other physiological inhibitors of thrombin in the absence of heparin include α2 macroglobulin and α1 antitrypsin.

Principles of Testing

Affinity-purified antibody to FII is coated onto the wells of a microtitre plate. The plates are washed and plasma or other fluids containing FII are applied. The coated antibody will capture the FII in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to FII is added to the plate to bind to the captured FII. 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 F.II present in the sample.

Reagents And Materials Provided

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

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)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Sample Diluent: HBS-BSA-T20
5. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
6. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
7. Stopping Solution: 2.5 M H2SO4
8. 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 2 hours at 22°C.
2. **Blocking:** Blocking is not required under the conditions described. Washing the plate with PBS-Tween is sufficient to block nonspecific interactions. Wash plate 3 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/5,000(100%) then serial 1/2's down to 1/160,000(3.13%). Sample plasmas are diluted 1/10,000, 1/20,000 and 1/40,000. 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 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.