Version 01-06/20

User's Manual

Human Fibrinogen Matched Antibody Pair

REF **ABPR-L001**

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 FGA

General Description

Fibrinogen is an abundant plasma protein (5-10 uM) produced in the liver. The intact protein has a molecular weight of 340 kDa and is composed of 3 pairs of disulphide-bound polypeptide chains named Aα, Bβ and γ. Fibrinogen is a triglobular protein consisting of a central E domain and terminal D domains. Proteolysis by thrombin results in release of Fibrinopeptide A (FPA, Aα1-16) followed by Fibrinopeptide B (FPB, Bβ1-14) and the fibrin monomers that result polymerize in a half-overlap fashion to form insoluble fibrin fibrils. The chains of fibrin are referred to as α, β and γ, due to the removal of FPA and FPB. The polymerised fibrin is subsequently stabilized by the transglutaminase activated Factor XIII that forms amide linkages between γ chains and, to a lesser extent, α chains of the fibrin molecules. Proteolysis of fibrinogen by plasmin initially liberates C-terminal residues from the Aα chain to produce fragment X (intact D-E-D, which is still clottable). Fragment X is further degraded to non-clottable fragments Y (D-E) and D. Fragment Y can be digested into its constituent D and E fragments. Digestion of non-crosslinked fibrin with plasmin is very similar to the digestion of fibrinogen, which results in production of fragments D and E. Degradation of crosslinked fibrin by plasmin results in fragment DD (D-Dimer consisting of the D domains of 2 fibrin molecules crosslinked via the γ chains), fragment E (central E domain) as well as DDE in which fragment E is non-covalently associated with DD. For human crosslinked fibrin, the relative weights of the cleavage fragments produced are: 184 kDa for fragment DD, 92 kDa for D, 50 kDa for E, 1.54 kDa for FPA and 1.57 kDa for FPB.

Principles of Testing

Affinity-purified polyclonal antibody to Fg is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with bovine serum albumin. The plates are washed and plasma or other fluids containing Fg are applied. The coated antibody will capture the Fg in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to Fg is added to the plate to bind to the captured Fg. 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 H2SO4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of Fg in the sample.

Reagents And Materials Provided

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

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: Adjust pH to 7.4, if necessary.
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 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 with wash buffer.
3. **Samples:** Reference plasma is diluted 1/10,000 (100%) then serial 1/2's down to 1/320,000 (13%). Sample plasmas are diluted 1/20,000, 1/40,000 and 1/80,000. 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 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 X 3 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.

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