Version 14-04/25

User's Manual

Human Factor XIII Matched Antibody Pair

REF **ABPR-L018**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human Factor XIII

General Description

Factor XIII (F.XIII, fibrin stabilizing factor) is the proenzyme form of a transamidase that is essential for normal haemostasis and fibrinolysis, wound healing, female fertility and foetal development. Extracellular F.XIII consists of A subunits (83 kDa each) which contain the enzyme moiety, and B subunits (76 kDa each) which act as a carrier protein for the A subunit in circulation. Both subunits are produced under separate genetic control. In plasma, F.XIII exists as a non-covalent tetrameric complex (320 kDa) of two A-subunits and two B-subunits (A2B2). The concentration of F.XIII tetramer in plasma is ~25 µg/ml (~80 nM). An intracellular form of F.XIII is found in platelets, megakaryocytes and monocytes. This form of F.XIII presents as a dimer of two A-subunits only and has a molecular weight of 160 kDa. The importance of these intracellular stores is demonstrated by the observation that platelets can contribute up to half of the F.XIII activity in platelet rich plasma. The activation of F.XIII involves several steps. Thrombin cleaves after Arg37 of each A-subunit in the A2B2 tetramer, releasing a 4.5 kDa activation peptide. Additional conformational changes induced by the binding of calcium, and by dissociation of the B-subunits from the A-subunit dimer are required to obtain full enzyme activity. F.XIIIa is a cysteine protease that catalyses the formation of γ-glutamyl-ε-lysyl bonds between the γ and α chains of polymerised fibrin molecules. Other proteins found crosslinked into fibrin clots by F.XIIIa include fibrinogen, α2 Antiplasmin, fibronectin, vitronectin and von Willebrand factor.

Principles of Testing

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

Reagents And Materials Provided

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

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 for 2 hours at ambient temperature 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 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/200(100%) then serial 1/2's down to 1/6400(3.13%). Sample plasmas are diluted 1/400, 1/800 and 1/1600. All dilutions are made in PBSTween 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 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.