Version 01-06/20

User's Manual

Human Prekallikrein (PK) Matched Antibody Pair

REF **ABPR-L017**

Σ **Sufficient reagent for 4 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

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 Prekallikrein (PK)

General Description

Prekallikrein (PK), previously known as Fletcher Factor, is a single chain glycoprotein produced in the liver. The plasma concentration of PK is 50 μg/ml (550 nM), approximately 75% of which circulates in complex with high molecular weight kininogen (HK) and the remainder as free PK. Plasma PK is heterogeneous in both mass and charge due to variable degrees of glycosylation. Approximately 90% of plasma PK has an apparent molecular weight of 88 kDa as determined by SDS-PAGE and the remaining 10% has an apparent mass of 85 kDa. The catalytic site resides in the light chain. The heavy chain of PK contains four apple-domain structures similar to those found in F.XI and these are required for binding of PK to HK. PK is the zymogen form of the enzyme kallikrein, which is involved in the proteolysis of kininogens with subsequent release of bradykinin, a potent vasodilator. PK participates in the contact phase of coagulation as a substrate for surface-bound activated factor XII (F.XIIa) in the presence of the cofactor HK. As PK and factor XI (F.XI) both circulate in complex with HK, both are localized to activating surfaces through their respective binding to HK. Limited proteolysis of PK by F.XIIa generates kallikrein, a two-chain serine protease that initiates the reciprocal activation of PK and F.XI. Kallikrein activity in plasma is regulated predominantly by C1-Inhibitor and α2 macroglobulin, with relatively minor contributions by Protein C Inhibitor, α2 Antiplasmin, and antithrombin.

Principles of Testing

Affinity-purified antibody to PK 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 PK are applied. The coated antibody will capture the PK in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to PK is added to the plate to bind to the captured PK. 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 H2SO4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of PK present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.4 ml of polyclonal affinity purified anti-PK antibody for coating plates.
2. Detecting Antibody (red): 0.4 ml of peroxidase-conjugated polyclonal anti-PK antibody for detection of captured PK.

Note: Reagents are sufficient for at least 4×96 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 and 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 1/500(100%) then serial 1/2's down to 1/16000(13%). Sample plasmas are diluted 1/1000, 1/2000 and 1/4000. All dilutions are made in HBSBSA-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 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 5-10 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.