Version 28-04/25

User's Manual

Human Kininogen (KN) Matched Antibody Pair

REF **ABPR-L016**

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 KNG1 (KN)

General Description

Kininogens are multi-function proteins that are involved in the processes of coagulation, anticoagulation, fibrinolysis, inflammation and cell adhesion. Kininogens are produced in the liver but have also been found in platelets, granulocytes, renal tubular cells and skin. Two forms of kininogen are identified in plasma, both of which are the result of differential splicing of a single gene. High molecular weight kininogen (HK), previously known as Fitzgerald Factor, is a single chain glycoprotein of 120 kDa with a plasma concentration of 80 μg/mL (660 nM). Low molecular weight kininogen (LK), also known as α-cysteine protease inhibitor, is a single chain glycoprotein of 68 kDa with a plasma concentration of 160 μg/mL (2.35 μM). HK and LK share a common heavy chain and bradykinin domain, but have unique light chains. It is the light chain of HK that is responsible for the coagulant cofactor activity by binding to anionic surfaces and for the ability to bind the zymogens prekallikrein (PK) and factor XI (F.XI). HK is cleaved by kallikrein in several sequential steps that result in the release of a potent vasodilator bradykinin and the conversion to a two-chain form of HK with increased cofactor activity. In plasma, most of the PK and F.XI circulate in complex with HK. Activation of PK by F.XIIa generates kallikrein, which initiates reciprocal activation of PK and F.XI. The presence of HK also serves to protect kallikrein and activated F.XI from protease inhibitors such as C1-Inhibitor, but regulation of the system may be accomplished through proteolytic inactivation of the HK cofactor activity by these enzymes.

Principles of Testing

Affinity-purified antibody to KN 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 KN are applied. The coated antibody will capture the KN in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to KN is added to the plate to bind to the captured KN. 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 H_2SO_4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of KN present in the sample.

Reagents And Materials Provided

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

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. 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 overnight at 2-8°C or 2hrs at 22°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 1/1000(100%) then serial 1/2's down to 1/32000(3.13%). Sample plasmas are diluted 1/2000, 1/4000 and 1/8000. All dilutions are made in PBS-Tween 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 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.