Version 17-04/25

User's Manual

Human Plasminogen Matched Antibody Pair

REF **ABPR-L031**

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 PLG

General Description

Plasminogen (Pg) is synthesized in the liver and circulates in plasma at a concentration of ~200 µg/ml (~2.3 µ M). Plasminogen is a single-chain glycoprotein of ~88 kDa that consists of a catalytic domain followed by five kringle structures. Within these kringle structures are four low-affinity lysine binding sites and one high-affinity lysine binding site. It is through these lysine binding sites that plasminogen binds to fibrin and to α2 Antiplasmin. Native plasminogen (glu-plasminogen) exists in two variants that differ in their extent of glycosylation, and each variant has up to six isoelectric forms with respect to sialic acid content, for a total of 12 molecular forms. Activation of glu-plasminogen by the plasminogen activators urokinase (UPA), or tissue plasminogen activator (tPA) occurs by cleavage after residue Arg560 to produce the two-chain active serine protease plasmin. In a positive feedback reaction, the plasmin generated cleaves an ~8 kDa peptide from glu-plasminogen, producing lys77-plasminogen which has a higher affinity for fibrin and when bound is a preferred substrate for plasminogen activators such as urokinase. Additional activators of plasminogen include kallikrein and activated factor XII. The primary inhibitor of plasmin in plasma is α2 Antiplasmin. Other physiological inhibitors of plasmin include α2 macroglobulin and antithrombin.

Principles of Testing

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

Reagents And Materials Provided

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

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. 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 for 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 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/10,000(100%) then serial 1/2's down to 1/320,000(3.13%). 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 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 5-10 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.