Version 26-04/24

User's Manual

Human Apolipoprotein-H (β2 Glycoprotein I) Matched Antibody Pair

REF **ABPR-L030**

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 APOH

General Description

Apolipoprotein-H, also known as β2-Glycoprotein I (β2GPI), is a plasma glycoprotein that circulates at a concentration of 200 ug/ml (4 μM). Synthesized in the liver, β2GPI is a single chain molecule of 48 kDa, consisting of five repeating internally disulphide-bonded structures referred to as sushi domains. Relative to other glycoproteins, β2GPI has an unusually high content of cysteine (6.2%), proline (8.3%) and carbohydrate (19%). Almost half the circulating β2GPI in plasma is associated with lipoproteins of all major fractions. β2GPI has been demonstrated to bind negatively charged phospholipids, heparin and platelets. Although the precise function(s) are as yet unknown, β2GPI has been demonstrated to interfere with blood coagulation by competitively binding to negatively charged phospholipid surfaces exposed during cell activation or damage. Recent evidence also implicates β2GPI as a cofactor recognized by anti-phospholipid antibodies present in some autoimmune disorders such as systemic lupus erythematosus (SLE).

Principles of Testing

Affinity-purified antibody to β2 GPI is coated onto the wells of a microtitre plate. The plates are washed and plasma or other fluids containing β2 GPI are applied. The coated antibody will capture the β2 GPI in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to β2 GPI is added to the plate to bind to the captured β2 GPI. 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 β2 GPI present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of polyclonal affinity-purified anti-β2 GPI antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase-conjugated polyclonal anti-β2 GPI antibody for detection of captured β2GPI.

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)

3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Sample Diluent: HBS-BSA-T20
5. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
6. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
7. Stopping Solution: 2.5 M H2SO4
8. 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:** Blocking is not required under the conditions described. Washing the plate with PBS-Tween is sufficient to block nonspecific interactions. Wash plate 3 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/2000(100%) then serial 1/2's down to 1/64000(3.13%). Sample plasmas are diluted 1/4000, 1/8000 and 1/16000. 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 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 wavelength of 490 nm.