Version 18-04/25

User's Manual

Human Heparin Cofactor II Matched Antibody Pair

REF **ABPR-L028**

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 SERPIND1

General Description

Heparin Cofactor II (HCII), also known as heparin cofactor A and dermatan sulphate cofactor, is a single chain glycoprotein produced in the liver and circulates in plasma at concentration of 50-100 μg/ml (0.8-1.5 μ M). A member of the SERPIN family of proteinase inhibitors, HCII exhibits a relatively narrow specificity and is only known to inhibit the serine proteinases thrombin and chymotrypsin. The inhibitory activity toward thrombin is accelerated up to 1000-fold in the presence of optimal concentrations of heparin. Unlike Antithrombin, thrombin inhibition by HCII is also enhanced by dermatan sulphate. HCII has an apparent molecular weight of 65,600 daltons and interaction with thrombin results in a covalent 1:1 enzyme-inhibitor complex of 102,600 daltons.

Principles of Testing

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

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.4 ml of polyclonal affinity purified anti-HCII antibody for coating plates.
2. Detecting Antibody (neutral): Four neutral-capped tubes each containing 10 ml of pre-diluted peroxidase conjugated polyclonal anti-HCII antibody for detection of captured HCII. Store reagents at 2-8°C.

Note: Reagents are sufficient for at least 4×96 well plates using recommended protocols.

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

2-8°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 4°C.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for a minimum of 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(3.13%). Sample plasmas are diluted 1/1000, 1/2000 and 1/4000. 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:** Apply the pre-diluted detecting antibody, 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.