Version 17-04/25

User's Manual

Murine Antithrombin Matched Antibody Pair

REF **ABPR-L026**

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 Murine SERPINC1

General Description

Antithrombin, also known as Antithrombin III (ATIII), is a member of the SERPIN family of proteinase inhibitors and the primary inhibitor of thrombin in plasma. It is produced in the liver and circulates in plasma at ~200 μg/ml (~3.5 μM). Antithrombin inhibits a broad spectrum of serine proteases including thrombin, activated forms of factor X, factor IX, factor XI, factor XII, as well as kallikrein, plasmin and urokinase. Enzyme inhibition by antithrombin occurs through proteolytic cleavage at Arg385-Ser386 and subsequent rapid formation of a stable, inactive 1:1 enzyme-antithrombin complex. Heparin has a profound accelerating effect on the inhibitory activity of antithrombin towards some enzymes. For example, the rate of inhibition of thrombin and activated factor X is increased 1000-fold in the presence of optimal concentrations of heparin, whereas heparin has relatively little effect on the inhibition rate of activated factor XI, activated factor XII and kallikrein. Antithrombin is a single chain molecule with a molecular weight of 59 kDa. Interaction with thrombin results in an SDS-stable thrombin-antithrombin complex of 96 kDa.

Principles of Testing

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

Reagents And Materials Provided

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

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 sample diluent)
3. Wash Buffer and Sample Diluent: PBS-Tween(0.1%, v/v)

4. Detecting Antibody 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 room temperature.
2. **Blocking:** Blocking is not required under the conditions described. Washing the plate with PBS-Tween is sufficient to block non specific interactions. Wash plate 3 times with wash buffer.
3. **Samples:** Normal mouse plasma is diluted 1/4,000(100%) then serial 1/2's down to 1/128,000(3.13%). Sample plasmas are diluted 1/8,000, 1/16,000 and 1/32,000. All dilutions are made in PBS-Tween. 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 wavelength of 490 nm.

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