Version 16-04/24

User's Manual

Human Activated Protein C-α1 Antitrypsin complex (APCAT) Matched Antibody Pair

REF **ABPR-L022**

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 Activated Protein C- α1Antitrypsin complex (APCAT)

Principles of Testing

Affinity-purified antibody to human Protein C is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with bovine serum albumin. The plates are washed and plasma or other fluids are applied. The coated antibody will capture the APC and APC-inhibitor complexes in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to α1 AT is added to the plate to bind to the captured APCAT complexes. 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 APCAT complex present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of polyclonal affinity purified anti-Protein C antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated polyclonal anti-α1 AT antibody for detection of captured APCAT complex.

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 and Detecting Antibody 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. Materials for making reference standards: Protein C deficient plasma; Human Activated Protein C; Human α1Antitrypsin; PPACK(Phe-Pro-Arg-CMK).
10. Other: Microplates; Microplate washer; Microplate reader.

Storage

-10 to -20°C

Assay Procedure

1. **Preparation of APCAT reference standard:** Purified APC(2 µg/mL = 33 nM) in 50 mM HEPES, 0.15 M NaCl, pH 7.4, is incubated with an excess of purified α1 AT(1.5 mg/mL = 27.3 µM) at 37°C. Complex formation is monitored by measuring residual APC activity using anticoagulant or chromogenic assay. When approximately half of the APC has been inhibited the reaction is quenched by the addition of PPACK to a final concentration of 50 µM to prevent further complex formation. The concentration of complex is calculated from the amount of APC activity inhibited by α1AT before the addition of PPACK. A series of standards can be made by diluting this stock APCAT complex into PC deficient plasma. The highest APCAT concentration should be 2.5 nM, serially diluted into PC-DP to obtain APCAT concentrations of 1.25, 0.625, 0.313, 0.156 and 0.078 nM respectively.
2. **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 or overnight at 2-8°C.
3. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 90 minutes at 22°C. Wash plate 3 times with wash buffer.
4. **Standards and Test Samples:** Test samples and the reference standards prepared above are each diluted 1/100 in HBS-BSA-T20 diluent. Apply 100 µl/well and incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
5. **Detecting Antibody:** Dilute the detecting antibody 1/100 in HBS-BSA-T20 detecting antibody diluent and apply 100 µl to each well. Incubate plate at 22°C for 60 minutes. Wash plate 3 times with wash buffer.
6. **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.