Creative Diagnostics®

Version 17-04/25

User's Manual

Human α1 Antitrypsin antigen (α1 AT) Matched Antibody Pair

REF **ABPR-L021**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human SERPINA1

General Description

Alpha 1 Antitrypsin (α1AT), also known as Alpha 1Proteinase inhibitor (α1PI), is the most abundant protease inhibitor in blood and a member of the SERPIN family of proteinase inhibitors. Serum levels are typically 1.3 mg/ml (25 μM) but α1AT is an acute phase protein and concentrations can rise four-fold during inflammatory episodes or tissue injury. Low levels in circulation have been associated with pulmonary disease such as emphysema. α1AT is a single chain molecule with a mass of 52,000 daltons that is produced primarily in the liver and to a lesser extent by blood monocytes and intestinal epithelium. Based on association rates, the primary target enzyme for α1AT is believed to be neutrophil elastase, but α1AT is a broad-spectrum inhibitor for many serine proteinases and the main role of α1AT in vivo is likely that of a "backup"inhibitor and proteinase scavenger in fluids and tissues. Although the association rates of α1AT with other enzymes are lower, the high concentration in plasma makes it an important inhibitor of activated Protein C, activated F.XI, thrombin and plasmin. Enzyme inhibition by α1AT occurs through proteolytic cleavage between Met358 and Ser359, which induces a conformational change in α1AT locking the enzyme into a stable, inactive 1:1 enzyme-inhibitor complex.

Principles of Testing

Affinity-purified antibody to α1 AT 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 α1 AT are applied. The coated antibody will capture the α1 AT 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 α1 AT. 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 α1 AT present in the sample.

Reagents And Materials Provided

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

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 2 hours at22°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 4 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/20,000(100%) then serial 1/2's down to 1/640,000(3.13%). Sample plasmas are diluted 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 4 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 4 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 and 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.