Version 28-04/25

User's Manual

Human Protein S (PS) Matched Antibody Pair

REF **ABPR-L023**

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 PROS1

General Description

Protein S (PS) is a vitamin K-dependent glycoprotein produced in the liver, endothelium and megakaryocytes. The concentration of PS in plasma is ~25 μg/ml (~325 nM) where it acts as a cofactor in the anticoagulant activity of activated Protein C. A deficiency of Protein S (quantitative or qualitative) is a risk factor for vascular thrombosis. Protein S is expressed as a single chain molecule with a molecular weight of 77 kDa. The structure of PS is similar to many other vitamin-K dependent coagulation proteins, consisting of an N-terminal calcium binding domain of 10 γ-carboxyglutamic acid (gla) residues, followed by a thrombin-sensitive loop region and 4 EGF-like domains. The C-terminal domain does not contain the usual catalytic triad of a proenzyme, but seems instead to be involved in the binding of PS to C4b-binding protein (C4bp). Protein S binds to activated Protein C (APC) in the presence of calcium and negatively charged phospholipid surface to allow APC to proteolytically inactivate coagulation cofactors Va and VIIIa. Enzymatic regulation of PS cofactor activity is through cleavage of PS in the thrombin-sensitive loop region by thrombin or other enzymes, resulting in the loss of calcium binding properties and APC cofactor activity. Another regulatory mechanism is to reduce the availability of PS by the binding of PS to C4bp. In plasma, approximately 60% of Protein S circulates in non-covalent complex with C4bp, making it unavailable for APC cofactor activity. The binding of PS to C4bp may be important in localizing C4bp to damaged cell membranes where it may control activation of complement by the classical pathway.

Principles of Testing

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

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of affinity-purified polyclonal anti-Protein S antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated polyclonal anti-Protein S antibody for detection of captured Protein S. 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 overnight at 2-8°C.
2. **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.
3. **Samples:** Total Protein S: Reference plasma is diluted 1/400(100%) then serial 1/2's down to 1/12,800(3.13%). Sample plasmas are diluted 1/800, 1/1,600 and 1/3,200. Free Protein S: Reference plasma and samples are PEG precipitated before use. Reference plasma supernatant is diluted 1/200(100%) then serial 1/2's down to 1/6,400(3.13%). Sample plasma supernatants are diluted 1/400, 1/800 and 1/1600. All dilutions are made in HBS-BSA-T20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 120 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 90 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 a wavelength of 490 nm.