



## Human Protein S Matched Pair Antibodies for EIA (5 x 96 Tests)

**REF** PS-EIA

For Research Use Only  
Not for Use in Diagnostic Procedures  
For in vitro use only

**Store at -10 to -20° C**

### INTENDED USE:

Human Protein S (PS) Matched Pair Antibodies for EIA are intended for use with in-house enzyme-linked immunosorbent assays for measuring human PS in plasma, or in any biological fluid where human PS can be present. The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

### SUMMARY:

Human Protein S (molecular weight about 77kDa) is a vitamin K-dependent plasma glycoprotein synthesized in the liver and composed of a single polypeptide chain. Protein S is a non-enzymatic cofactor to APC for the inactivation of FVa and FVIIIa. Total Protein S concentration in human plasma is about 25 µg/mL (325 µM). About 40% of plasma Protein S exists in free form, and 60% is non-covalently complexed with C4b Binding Protein (C4BP). Complex formation does not lead to inactivation of C4BP, although Protein S no longer functions as a cofactor.

### ASSAY PRINCIPLE:

The diluted plasma sample or biological fluid is introduced into one of the microwells of a micro-ELISA plate which has been pre-coated with anti-human PS antibody. When present in the added material, PS binds to the anti-human antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human peroxidase conjugate, which reacts specifically with human PS. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), is introduced and a yellow color develops. The color turns orange when the reaction is stopped with sulfuric acid. The color developed is directly proportional to the amount of PS present in the tested sample.

### REAGENTS:

*Required Materials provided (enough for 5x96 Tests):*

- **C: Capture Antibody (PS-EIA-C).** 1 vial of 0.5 polyclonal antibody specific for human PS. For coating plates. Yellow cap.
- **D: Detecting Antibody (PS-EIA-D).** 1 vial of 0.5 mL polyclonal antibody specific for human PS, coupled to peroxidase. For detecting captured PS. Red cap.

**Note:** Antibodies are provided in a glycerol solution (50% v/v) and should be **stored at -10 to -20°C**. Vials should be tightly capped. Do not store in frost-free freezers

Antibodies can be centrifuged briefly in a micro-centrifuge to gather residual reagent from the cap and walls of the tube.

In their original packaging, before use, when stored at -10 to -20°C, the unopened antibodies are stable until the expiration date printed on the vial.

### Required Materials not provided:

Optimum performance can be obtained when the following solutions and assay conditions are used.

- **Micro ELISA plates** with hydrophilic surface designed for high binding of IgG. For example, 96-well Immulon 4-HBX.
- **Coating Solution** (50mM Carbonate). Dissolve 1.59 g of Na<sub>2</sub>Cl<sub>3</sub> and 2.93 g of NaHCO<sub>3</sub> in distilled water to a final volume of 1 L and adjust pH to 9.6. Store at 2-8°C for 1 month.
- **Phosphate-Buffered Saline (PBS)** [For preparation of sample diluent, wash and blocking solutions.] Dissolve 8.0 g NaCl, 1.15 g Na<sub>2</sub>HPO<sub>4</sub>, 0.2 g KH<sub>2</sub>PO<sub>4</sub> and 0.2 g KCl in distilled water to a final volume of 1 L and adjust pH to 7.4. Store up to 1 month at 2-8°C, discard if there is evidence of microbial growth.
- **Wash Solution:** (PBS/Tween-20 0.1% v/v). Add 1.0 mL of Tween-20 to 1 L of PBS and adjust pH to 7.4. Store at 2-8°C up to 1 week.
- **Blocking Solution:** (PBS/BSA 1% w/v). Dissolve 2.5 g of Bovine Serum Albumin in 200 mL of PBS and adjust pH to 7.4; add PBS to final volume of 250 mL. Aliquot and store frozen at -20°C.
- **Sample Diluent:** (HEPES/BSA/Tween-20). Dissolve 5.95 g HEPES (free acid), 1.46 g NaCl, and 2.5 g Bovine Serum Albumin (Sigma, RIA grade) in 200 mL distilled H<sub>2</sub>O; add 0.25 mL of Tween-20 and adjust pH to 7.2 with NaOH; add distilled water to final volume of 250 mL. Aliquot and store frozen at -20°C for up to 6 months.
- **Substrate Solution:** (Citrate-Phosphate buffer). Dissolve 2.6 g Citric Acid and 6.9 g Na<sub>2</sub>HPO<sub>4</sub> in 450 mL distilled H<sub>2</sub>O and adjust pH to 5.0 with Phosphoric acid or NaOH; add distilled water to final volume of 500 mL. Store at 2-8°C up to 1 month.
- **OPD Substrate** (o-phenylenediamine.2HCl) ☒ ToXc! 5 mg tablets: e.g. Sigma #P-6912. Prepare immediately before use. Dissolve 5 mg OPD in 12 mL Substrate Solution and then add 12 µL 30% H<sub>2</sub>O<sub>2</sub>. Do not store.
- **Stop Solution:** (2.5M H<sub>2</sub>SO<sub>4</sub>) ☒ Corrosive! Generates heat on dilution! Handle with great care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling. Carefully add 13.9 mL 18M H<sub>2</sub>SO<sub>4</sub> to 86 mL distilled H<sub>2</sub>O. Store at room temperature for up to 1 month.
- **Reference standards** for Protein S which have the same matrix and anticoagulant as the samples to be tested
- Micro ELISA plate washing equipment and shaker.
- Plate reader with a wavelength set up at 490 nm.

### PROCEDURE:

**1. Coat ELISA plate:** Dilute the Capture Antibody with Coating Solution 1/100 (use polypropylene tube) and immediately add

100  $\mu$ L to every well in the plate. Incubate 2 hours at 22°C or preferable overnight at 2-8°C.

**2. Blocking:** Empty contents of plate and add 150  $\mu$ L of Blocking Solution to every well and incubate for 90 minutes at 22°C. This step blocks any remaining binding sites on the plastic wells. Wash plate 3X with Wash Solution.

### 3. Samples:

TOTAL Protein S: Dilute PS Reference standard with Sample Diluent 1/400 (100%) then serially dilute by halves down to 1/12,800 (3.13%). Dilute sample plasmas or biological fluid with Sample Diluent 1/800, 1/1,600 and 1/3,200.

FREE Protein S: Reference plasma and samples are PEG precipitated before use (Comp PC et al, Blood 67, pp 504-508, 1986). Reference standard supernatant is diluted 1/200 (100%) then serially dilute by halves down to 1/6,400 (3.13%). Sample plasma supernatants are diluted with Sample Diluent 1/400, 1/800 and 1/1,600.

Apply 100  $\mu$ L per well and incubate plate at 22°C for 120 minutes. Wash plate 3X with Wash Solution. (Plasma samples should not be applied at dilutions lower than 1/20, as falsely high readings may result.)

**4. Detecting Antibody:** Dilute the Detecting Antibody with Sample Diluent 1/100 and apply 100  $\mu$ L to each well. Incubate plate at 22°C for 90 minutes. Wash plate 3X with Wash Solution.

**5. OPD Substrate:** Apply 100  $\mu$ L of freshly prepared OPD substrate to each well. Allow color to develop for 5-10 minutes then stop color reaction with the addition of 50  $\mu$ L per well of Stop Solution. Read the plate at a wavelength of 490 nm. [Optimal color development time is the time required to obtain A490  $\geq$  1.000 for the 100% reference point, not to exceed 20 minutes.]

### Additional Notes:

- Do not shake plate during incubation.
- Do not allow the wells to become dry. Keep plate covered or in a humid chamber during incubations.
- Rheumatoid factor in samples may bind to the capture and/or detecting antibodies and cause interference in the ELISA assay.

**6. Calibration Curve:** On bi-logarithmic graph paper, plot the known Protein S concentrations on abscissa and the corresponding absorbance (A490) on ordinates in order to establish the calibration curve.

### RESULTS:

From the constructed calibration curve, directly determine the Protein S concentration and multiply by the appropriate dilution factor.

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