5-DIAGNOSTICS

Human Factor V
Matched Pair Antibodies for EIA
(5 x 96 Tests)

Ref#: 5D-18116
Lot#: XXX
Exp Date: XXX

Store at -10 to -20°C

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

Last Revision: 2014/02/06

INTENDED USE:

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

SUMMARY:

Coagulation Factor V (FV) is a 320 kDa glycoprotein synthesized in the liver. FV is activated through limited proteolysis by thrombin or by FXa in the presence of phospholipids. FV may also be activated to FVa by plasmin, neutrophil elastase or platelet calpain. The activated FV is an essential cofactor of the prothrombin activator complex, which consists of FVa, FXa, calcium and anionic phospholipid surface. The normal Factor V concentration in human plasma is about 10 µg/mL.

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 polyclonal anti-human FV antibody. When present in the added material, FV binds to the antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human peroxidase conjugate, which reacts specifically with human FV. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H_2O_2), 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 FV present in the tested sample.

REAGENTS:

Required Materials provided (enough for 5x96 Tests):

C: Capture Antibody (F5-1099P-C). 1 vial of 0.5 mL purified polyclonal antibody specific for human FV. For coating plates. Supplied in a 50% v/v glycerol solution. Yellow cap.

D: Detecting Antibody (F5-1099P-D). 1 vial of 0.5 mL polyclonal antibody specific for human FV, coupled to peroxidase. For detecting captured FV. Supplied in a 50% v/v glycerol solution. 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**. 50 mM Carbonate. Dissolve 1.59 g of Na2Cl3 and 2.93 g of NaHCO3 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 wash solution and blocking solutions.] Dissolve 8.0 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4 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 (Sigma-RIA grade) 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 and Conjugate 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_2O; 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.
- **Substrate Solution:** Citrate-Phosphate buffer. Dissolve 2.6 g Citric Acid and 6.9 g Na_2HPO_4 in distilled water up to a final volume of 500 mL and adjust **pH to 5.0**. Store at 2-8°C up to 1 month.
- **OPD Substrate**: o-phenylenediamine.2HCl ☒ Toxic! (5 mg tablets: Sigma #P-6912). Prepare immediately before use. Dissolve 5 mg OPD in 12 mL Substrate Solution and then add 12 µL 30% H_2O_2. Do not store.
- **Stop Solution: 2.5 M H_2SO_4**. ☒ 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 18 M H2SO4 **to** 86 mL distilled H_2O. Store at room temperature.
- **Reference standards** for Factor V which have the same matrix and anticoagulant as the samples to be tested.
- Micro ELISA plate washing equipment and shaker.
- Micro ELISA 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 µL to every well in the plate. Incubate overnight @ 4°C.

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

3. Samples: Dilute FV Reference standard with Sample Diluent 1/200 (100%) then serially dilute by halves down to 1/6400 (3.13%). Dilute sample plasmas or biological fluid with Sample Diluent 1/400, 1/800 and 1/1600. Apply 100 µL per well and incubate plate @ 22°C for 90 minutes. Wash plate 3X with Wash Solution. [Plasma samples should not be applied at dilutions lower than 1/10, as falsely high readings may result.]

4. Detecting Antibody: Dilute the Detecting Antibody with Sample/Conjugate Diluent 1/100 and apply 100 µL to each well. Incubate plate @ 22°C for 90 minutes. Wash plate 3X with Wash Solution.

5. OPD Substrate: Apply 100 µL of freshly prepared OPD substrate to each well. Allow color to develop for 10-15 minutes then stop color reaction with the addition of 50 µL 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 ≥ 1.000 for the 100% reference point, not to exceed 20 minutes.]

Additional Notes:

- 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 Factor V 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 Factor V concentration and multiply by the appropriate dilution factor.

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