5-DIAGNOSTICS

Anti-human Factor VIII:C, Matched Pair Antibodies for EIA

REF 5D-18119

For Research Use Only.
Not for Use in Diagnostic Procedures.

Store at 2-8°C
4 x 96 Tests

INTENDED USE

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

SUMMARY

Coagulation Factor VIII (FVIII) is a 320 kDa glycoprotein synthesized in the liver. Factor VIII is stabilized by von Willebrand Factor (vWF). Thrombin-activated FVIII dissociates from vWF and combines with FIXa, calcium and phospholipids a cofactor in the assembly of the FX activator complex. The normal Factor VIII concentration in human plasma is about 200 ng/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 anti-human Factor VIII antibody. When present in the added material, Factor VIII binds to the anti-human polyclonal antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human peroxidase conjugate, which reacts specifically with human Factor VIII. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H_2O_2), is introduced and a yellow colour develops. The colour turns orange when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of Factor VIII present in the tested sample.

REAGENTS

*Required Materials (**provided**); enough for 4x96 Tests:*

C: Capture Antibody (5D-18119-C). 1 vial of 0.4 mL polyclonal affinity purified antibody specific for human Factor VIII. For coating plates. Yellow cap.

D: Detecting Antibody (5D-18119-D). 4 vials of 10 mL pre-diluted polyclonal antibody specific for human Factor VIII, coupled to peroxidase. For detecting captured Factor VIII. Neutral cap.

SD: Sample Diluent (5D-18119-SD). 1 vial of 100 mL diluent buffer for sample dilution. Green coloured solution.

The capture antibody 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 2 to 8°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.] 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.
- **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 H_2SO_4 **to** 86 mL distilled H_2O. Store at room temperature.
- **Reference standards** for Factor VIII 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 for 2 hours at 22°C.

2. Washing: Empty contents of plate. Wash plate 3X with Wash Solution.

3. Samples: Dilute Factor VIII Reference standard with Sample Diluent (Reagent **SD**) 1/4 (100%) then serially dilute by halves down to 1/256 (1.56%). Dilute sample plasmas or biological fluid with Sample Diluent 1/8, 1/16 and 1/32. Apply 100 µL 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 1/2, as falsely high readings may result.]

4. Detecting Antibody: Apply 100 µL to each well. Incubate plate at 22°C for 60 minutes. Wash plate 3X with Wash Solution.

5. OPD Substrate: Apply 100 µL of freshly prepared OPD substrate to each well. Allow colour to develop for 10-15 minutes then stop

colour reaction with the addition of 50 µL per well of Stop Solution. Read the plate at a wavelength of **490 nm.** [Optimal colour 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 VIII 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 VIII concentration and multiply by the appropriate dilution factor.

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