Anti-human Factor XII, Matched Pair Antibodies for EIA

REF 5D-18127

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

Store at -10 to -20° C
5 x 96 Tests

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

SUMMARY:
Coagulation Factor XII (FXII) is a 76 kDa protein synthesized in the liver. Cleavage by kallikrein after residure Arg_{353} produces the enzyme αFXIIa consisting of a 28 kDa light chain containing the protease domain and a 52 kDa heavy chain containing the anionic surface-binding domain. Further cleavage of αFXIIa by kallikrein produces the 28 kDa fragment βFXIIa. The activity of FXIIa is regulated by C1-Inhibitor with minor contributions by α2-antiplasmin, α2-macroglobulin and antithrombin. The normal Factor XII protease zymogen concentration in human plasma is about 30 µg/mL (400 nM).

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 FXII antibody. When present in the added material, FXII 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 FXII. 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 FXII present in the tested sample.

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

C: **Capture Antibody (5D-18127-C)**. 1 vial of 0.5 mL polyclonal affinity purified antibody specific for human FXII. For coating plates. Supplied in a 50% v/v glycerol solution. **Yellow cap.**

D: **Detecting Antibody (5D-18127-D)**. 1 vial of 0.5 mL polyclonal antibody specific for human FXII, coupled to peroxidase. For detecting captured FXII. 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 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 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 XII 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 or overnight at 2-8°C.

2. Blocking: Empty contents of plate and add 150 µL 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: Dilute FXII Reference standard with Sample Diluent 1/500 (100%) then serially dilute by halves down to 1/16000 (3.13%). Dilute sample plasmas or biological fluid with Sample Diluent 1/1000, 1/2000 and 1/4000. Apply 100 µL per well and incubate plate at 22°C for 60 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 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 5-10 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 XII 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 XII concentration and multiply by the appropriate dilution factor.

5-DIAGNOSTICS

5-Diagnostics AG
Heuberg 7, 4051 Basel,
Switzerland
www.5-diagnostics.com
info@5-diagnostics.com