Human Factor II (Prothrombin) Matched Pair Antibodies for EIA

(5 x 96 Tests)

REF F2-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 Factor II Matched Pair Antibodies for EIA are intended for use with in-house enzyme-linked immunosorbent assays for measuring human Factor II in plasma, or in any biological fluid where human Factor II can be present. The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

SUMMARY:

Coagulation Factor II (Prothrombin) is a 72 kDa glycoprotein synthesized in the liver and composed of a single polypeptide chain. Prothrombin is the zymogen form of Thrombin. Prothrombin is cleaved to produce thrombin by the membrane-bound prothrombinase complex (activated Factor X, Activated Factor V and Calcium). Prothrombin concentration in human plasma is about 100 µg/mL (1.4 µM)

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 II antibody. When present in the added material, Factor II binds to the anti-human polyclonal antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human Factor II peroxidase conjugate, which reacts specifically with human Factor II. 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 Factor II present in the tested sample.

REAGENTS:

Required Materials provided (enough for 5x96 Tests):

- **C: Capture Antibody (F2-EIA-C)**. 1 vial of 0.5 mL polyclonal affinity-purified antibody specific for Factor II. For coating plates. Yellow cap.
- **D: Detecting Antibody (F2-EIA-D)**. 1 vial of 0.5 mL polyclonal antibody specific for human Factor II, coupled to peroxidase. For detecting captured FII. 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_2Cl_3 and 2.93 g of $NaHCO_3$ 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.
- **Sample Diluent:** (HEPES/BSA/Tween-20). Dissolve 5.95 g HEPES (free acid), 1.46 g NaCl and 2.5 g Bovine Serum Albumin 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 for up to 6 months.
- **Substrate Solution: (**Citrate-Phosphate buffer). Dissolve 2.6 g Citric Acid and 6.9 g Na_2HPO_4 in 450 mL distilled H_2O 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) ☒ Toxic! 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_2O_2. Do not store.
- **Stop Solution:** (2.5M 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 18M H_2SO_4 to 86 mL distilled H_2O. Store at room temperature for up to 1 month.
- **Reference standards** for Factor II 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 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 Prothrombin (Factor II) Reference standard with Sample Diluent *1/5,000 (100%)* then serially dilute by halves down to *1/160,000 (3.13%)*. Dilute sample plasmas or biological fluid with

Sample Diluent *1/10,000, 1/20,000 and 1/40,000*. 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 *1/50*, 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 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 II 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 II concentration and multiply by the appropriate dilution factor.

CoaChrom Diagnostica GmbH | Your Coagulation Experts
Hauptstrasse 5
2344 Maria Enzersdorf, Austria
Phone: +43-1-236 222 1 | Fax: +43-1-236 222 111
Phone: 0800-246 633 0 | Fax: 0800 - 246 633 3 (Toll free)
info@coachrom.com | www.coachrom.com