



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

REF F9-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 IX Matched Pair Antibodies for EIA are intended for use with in-house enzyme-linked immunosorbent assays for measuring human Factor IX in plasma, or in any biological fluid where human Factor IX can be present. The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

SUMMARY:

Coagulation Factor IX (FIX), also known as Christmas Factor, is a 55 kDa vitamin K-dependent glycoprotein synthesized in the liver and composed of a single polypeptide chain. Factor IX is present in plasma as a zymogen and, when activated to FIXa by Factor XIa in the presence of calcium, thrombin and phospholipids, it forms an active complex with thrombin-activated FVIII:C, which is then able to convert FX into FXa. Factor IX may also be activated by the Factor VII-Tissue Factor complex. The normal Factor IX concentration in human plasma is about 4-5 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 anti-human Factor IX antibody. When present in the added material, Factor IX binds to the anti-human polyclonal antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human Factor IX peroxidase conjugate, which reacts specifically with human Factor IX. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H₂O₂), 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 IX present in the tested sample.

REAGENTS:

Required Materials provided (enough for 5x96 Tests):

- **C: Capture Antibody (F9-EIA-C).** 1 vial of 0.5 mL polyclonal affinity-purified antibody specific for Factor IX. For coating plates. Yellow cap.
- **D: Detecting Antibody (F9-EIA-D).** 1 vial of 0.5 mL polyclonal antibody specific for human Factor IX, coupled to peroxidase. For detecting captured FIX. 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 Na₂CO₃ 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 Na₂HPO₄, 0.2 g KH₂PO₄ 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/EDTA/Tween-20). Dissolve 5.95 g HEPES (free acid), 1.46 g NaCl, 0.93 g Na₂EDTA, and 2.5 g Bovine Serum Albumin in 200 mL distilled H₂O; 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 Na₂HPO₄ in 450 mL distilled H₂O 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% H₂O₂. Do not store.
- **Stop Solution: (2.5M H₂SO₄)** ☒ 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 H₂SO₄ to 86 mL distilled H₂O. Store at room temperature for up to 1 month.
- **Reference standards** for Factor IX 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 Factor IX Reference standard with Sample Diluent 1/100 (100%) then serially dilute by halves down to 1/3,200

(3.13%). Dilute sample plasmas or biological fluid with Sample Diluent 1/200, 1/400 and 1/800. Apply 100 μ L per well and incubate plate at 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 Diluent 1/100 and apply 100 μ L to each well. Incubate plate at 22°C for 90 minutes. Wash plate 3X with Wash Solution.

5. OPD Substrate: Apply 100 μ 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 μ 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 Factor IX 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 IX concentration and multiply by the appropriate dilution factor.

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