Version 04-05/25

User's Manual

Human Factor IX Matched Antibody Pair

REF **ABPR-L010**

Σ **5 x 96 tests**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human Factor IX

General Description

Factor IX (F.IX, Christmas Factor) is a vitamin K-dependent glycoprotein produced in the liver. Plasma concentration of F.IX is normally around 5 μg/ml (87 nM) in plasma. The biological importance of F.IX is demonstrated in Haemophilia B (Christmas disease), an X-linked congenital bleeding disease resulting from a quantitative (low activity and low antigen) or qualitative (low activity and normal antigen) defect in F.IX function. In its proenzyme or zymogen form F.IX is a single chain molecule of 55,000 daltons. It contains two EGF-like domains and an amino-terminal domain containing 12 γ-carboxy-glutamic acid (Gla) residues. These Gla residues allow F.IX to bind divalent metal ions and participate in calcium-dependent binding interactions. The activation of F.IX occurs by limited proteolysis in the presence of calcium by activated factor XI (F.XIa) and/or by a complex of VIIa/tissue factor/phospholipid and activated Factor X between residues Arg146-Ala147 and between Arg180-Val181. The terminal activated product in either case is F.IXaβ, a two-chain enzyme consisting of a heavy chain (28,000 daltons), a light chain (18,000 daltons) and an activation peptide product of 11,000 daltons. F.IX can also be cleaved into inactive products by thrombin and by elastase. The activity of F.IXaβ in plasma is inhibited by antithrombin and this inhibition is accelerated 1000-fold in the presence of optimal concentrations of heparin.

Principles of Testing

Affinity-purified antibody to F.IX is coated onto the wells of a microtitre plate. The plates are washed and plasma or other fluids containing F.IX are applied. The coated antibody will capture the F.IX in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to F.IX is added to the plate to bind to the captured F.IX. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of H_2SO_4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of F.IX present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of polyclonal affinity purified anti-F.IX antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase-conjugated polyclonal anti-F.IX antibody for detection of captured F.IX.

Note: Reagents are sufficient for at least 5×96 well plates using recommended protocols. Antibodies are supplied in a 50% (v/v) glycerol solution for storage at –10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

Materials Required But Not Supplied

1. Coating Buffer: 50 mM Carbonate
2. PBS:(base for wash buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Sample Diluent: HBS-BSA-EDTA-T20
5. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
6. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
7. Stopping Solution: 2.5 M H2SO4
8. Other: Microplates; Microplate washer; Microplate reader.

Storage

-10 to -20°C

Assay Procedure

1. **Coating of plates:** Dilute the capture antibody 1/100 in coating buffer(preferably in a polypropylene tube) and immediately add 100 µl to every well in the plate. Incubate 2 hours at 22°C.
2. **Blocking:** Blocking is not required under the conditions described. Washing the plate with PBS-Tween is sufficient to block non specific interactions. Wash plate 3 times with wash buffer.
3. **Samples:** Reference plasma is diluted 1/100(100%) then serial 1/2's down to 1/3200(3.13%). Sample plasmas are diluted 1/200, 1/400 and 1/800. All dilutions are made in HBS-BSA EDTA-T20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 90 minutes. Wash plate 3 times with wash buffer.
4. **Detecting Antibody:** Dilute the detecting antibody 1/100 in HBS-BSA-EDTA-T20 sample diluent and apply 100 µl to each well. Incubate plate at 22°C for 90 minutes. Wash plate 3 times with wash buffer.
5. **OPD Substrate:** Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 5-10 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at wavelength of 490 nm.