Version 24-04/25

User's Manual

Human Factor VIII Matched Antibody Pair

REF **ABPR-L007**

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 VII

General Description

Factor VIII (formerly referred to as antihemophilic globulin and Factor VIII:C) is a large glycoprotein (320 kDa) that circulates in plasma at approximately 200 ng/ml. Synthesized in the liver, the majority of Factor VIII is cleaved during expression, resulting in a heterogeneous mixture of partially cleaved forms of F.VIII ranging in size from 200-280 kDa. The F.VIII is stabilized by association with von Willebrand Factor to form a F.VIII-vWF complex required for the normal survival of F.VIII in vivo (t1/2 of 8-12 hours). F.VIII is a pro-cofactor that is activated through limited proteolysis by thrombin. In this process F.VIIIa dissociates from vWF to combine with activated Factor IX, calcium and a phospholipid surface where it is an essential cofactor in the assembly of the Factor X activator complex. Once dissociated from vWF, F.VIIIa is susceptible to inactivation by activated Protein C and by non-enzymatic decay. Hemophilia A is a congenital bleeding disorder resulting from an X-chromosome-linked deficiency of F.VIII. The severity of the deficiency generally correlates with the severity of the disease. Some Hemophiliacs (~10%) produce a F.VIII protein that is partially or totally inactive. The production of neutralizing antibodies to F.VIII also occurs in 5-20% of Hemophiliacs.

Principles of Testing

Affinity-purified antibody to FVIII is coated onto the wells of a microtitre plate. The plate is washed and plasma or other fluids containing FVIII are applied. The coated antibody will capture the FVIII in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to FVIII is added to the plate to bind to the captured FVIII. 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.VIII present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.4 ml of polyclonal affinity purified anti-FVIII antibody for coating plates.
2. Detecting Antibody (neutral): Four neutral-capped tubes each containing 10 ml of pre-diluted peroxidase conjugated polyclonal anti-FVIII antibody for detection of captured FVIII.
3. Sample Diluent (green): 100 ml bottle containing a green-coloured diluent optimised for dilution of samples. Store reagents at 2-8°C

Note: Reagents are sufficient for at least 4×96 well plates using recommended protocols.

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. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
5. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
6. Stopping Solution: 2.5 M H2SO4
7. Other: Microplates; Microplate washer; Microplate reader.

Storage

2-8°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 1/4(100%) then serial 1/2's down to 1/256(1.56%). Sample plasmas are diluted 1/8, 1/16 and 1/32. All dilutions are made in the provided green sample diluent. Apply 100 µl/well and incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
4. **Detecting Antibody:** Apply the pre-diluted detecting antibody, 100 µl to each well. Incubate plate at 22°C for 60 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 10-15 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.