Version 29-04/24

User's Manual

Human von Willebrand Factor Matched Antibody Pair

REF **ABPR-L009**

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 VWF

General Description

von Willebrand Factor (vWF, also previously referred to as Factor VIII related antigen) is a large adhesive protein produced in endothelial cells and megakaryocytes. There are two critical functions of vWF, the first being its involvement in the process of platelet adhesion and aggregation through interaction with platelet receptor glycoprotein Ib, the second being the binding and stabilization of Factor VIII (antihemophilic factor) for secretion and transport in plasma. The vWF precursor protein is synthesized with a 95,000 dalton propeptide (also known as vWF antigen-II), believed to be involved in the intracellular multimerization of the vWF subunits. The mature vWF multimers are then packed into storage organelles within the cell (Weibel-Palade bodies) after which the propeptide is cleaved and released. vWF circulates as multimers of disulphide linked 220,000 dalton subunits and the molecular weight of these multimers ranges from 0.5-20 million daltons. The plasma concentration of vWF is typically 10 µg/ml, but increased levels are often observed in pregnancy and other conditions of physiological stress. von Willebrand's disease (vWD) is perhaps the most common inherited bleeding disorder in humans and is the result of either quantitative deficiencies of vWF (vWD Types I & III), or one of a number of qualitative disorders of vWF structure and function (vWD Type II).

Principles of Testing

Affinity-purified antibody to vWF is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing vWF are applied. The coated antibody will capture the vWF in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to vWF is added to the plate to bind to the captured vWF. 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 vWF present in the sample.

Reagents And Materials Provided

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

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 and blocking buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Blocking Buffer: PBS-BSA(1%, w/v)
5. Sample Diluent: HBS-BSA-T20
6. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
7. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
8. Stopping Solution: 2.5 M H2SO4
9. 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 or overnight at 2-8°C.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 minutes at 22°C. 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-BSAT20 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-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 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 a wavelength of 490 nm.

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