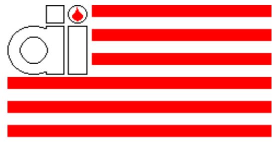


american diagnostica inc.

500 West Avenue, P.O. Box 110215 • Stamford, CT. 06911-0215
Tel. (203) 602-7777 Fax. (203) 602-2221
www.americandiagnostica.com

IMUBIND® vWF ELISA Kit

Product No. 828

INTRODUCTION

The IMUBIND® vWF ELISA kit is an enzyme-linked immunoassay for the quantitation of vWF antigen in human plasma.

This assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

BACKGROUND

Von Willebrand Factor (vWF) is a large, multimeric protein (molecular weight of 1,000-20,000 kD) composed of repeating 270 kD subunits containing 2050 amino acid residues. vWF is synthesized by endothelial cells and megakaryocytes, and is present in multimeric form in the basement membrane of the subendothelium, in plasma and platelets. The half-life of vWF in plasma is approximately 20 hours. Degraded forms of vWF are excreted in urine.[1,2] vWF functions as a carrier protein for Factor VIII, the coagulation protein absent in haemophilia A. It promotes platelet adhesion to damaged endothelium and participates in the platelet to platelet cohesion necessary for thrombus formation. Together with fibronectin and collagen, vWF functions in maintaining vessel wall integrity. Since vWF is synthesized in endothelial cells, it has been used as a marker for endothelial cell function and integrity. Measurements of vWF have been applied in a large number of basic investigations on endothelial cell function.

Patients with severe von Willebrand disorder (classified as type III) suffer from a complete absence of vWF in their plasma and urine. Patients with decreased circulating levels of vWF suffer from milder forms of the disorder, classified as type I, type IIa and type IIb.[2,3] vWD type I is a common disorder[4].

Studies have reported that in large vessel lesions of the endothelium such as arteriosclerosis, vWF levels have increased. However, in other studies, no significant increase of the factor has been observed[5]. Increased levels of the vWF/Factor VIII complex have also been reported in postoperative patients and patients with thrombosis[6].

It has been demonstrated that the determination of the plasma level of vWF:Ag is of value in the diagnosis, prognosis and monitoring of therapy in systemic vasculities and also for detecting latent vasculopathy[7,8]. Therefore, monitoring vWF levels may be important in selecting those patients with systemic arthritis who require early cytotoxic therapy. In addition, high levels of vWF have been shown to be an indicator of poor prognosis in patients with septicemia[9] and is considered to be a valuable marker of distant organ injury in patients having suffered skin burns.

vWF levels were measured in plasma and urine samples from patients with Type I diabetes mellitus (insulin-dependent) with and without signs of microangiopathy (retinopathy and nephropathy)[5]. Plasma vWF levels were significantly higher in all groups of patients compared to control patients, while urinary vWF levels were significantly higher in patients with microangiopathy. Qualitative differences in the excreted forms of vWF were observed only in those patients with clinical signs of nephropathy, where there was a shift towards excretion of the high molecular weight fragments.

PRINCIPLE

The IMUBIND vWF ELISA is a "sandwich" ELISA using a goat polyclonal antibody as the capture antibody. Samples incubate in precoated micro-test wells and the same polyclonal antibody, horseradish peroxidase (HRP) conjugated, is used to detect the bound vWF antigen. The addition of perborate/3,3',5,5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with the HRP, creates a blue colored solution. Sensitivity is enhanced by the addition of a 0.5M sulfuric acid stop solution, yielding a yellow colored solution. vWF levels are determined by measuring and comparing the absorbance of sample solutions at 450 nm against those of a standard curve developed using calibrated antigen.

REAGENTS

6 x 16 well precoated micro-test strips with holder and lid
6 vials vWF standards, 0 - 10 mU/mL (lyophilized)
1 vial Detection Antibody, HRP-conjugated anti-human vWF (135 µL)
1 vial Detection Antibody Diluent (lyophilized)
1 vial Substrate, TMB (11 mL)
1 packet Wash Buffer, PBS with 0.05% Tween 20, pH 7.4

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Distilled H_2O
50-200 µL eight channel multi-pipette
10-200 µL single pipette
Micro-test plate reader at 450 nm
0.5M H_2SO_4
Bovine Serum Albumin (BSA, e.g. Sigma A-7030)

REAGENT PREPARATION

A. Standards

1. Add 1.0 mL distilled H_20 to the 0.5, 1.0, 2.0, 5.0 and 10.0 mU/mL standard vials.
2. Add 2.0 mL distilled H_20 to the 0 mU/mL standard vial.
3. Agitate gently. Do not shake!

B. Detection Antibody Diluent

1. Add 20 mL of distilled H_2O to the Detection Antibody Diluent vial.
2. Mix well.

D. Wash Buffer

1. Dissolve the contents of the Wash Buffer packet in 900 mL distilled H_2O.
2. QS. to a final volume of 1 Liter with distilled H_2O.
3. Mix well and confirm pH is 7.4 (adjust if necessary).

E. Sample Buffer

Prepare an appropriate amount of Sample Buffer by adding BSA to Wash Buffer to a final concentration of 3% w/v (3 gm BSA/100 mL Wash Buffer).

REAGENT STABILITY

Store unused micro-test strips and unreconstituted reagents at +2-+8°C until the expiration dates indicated on their labels.

Aliquot and freeze reconstituted standards at -20°C. Store reconstituted reagents at +4°C for up to one month.

SAMPLE PREPARATION

1. Collect blood into 3.8% trisodium citrate anticoagulant solution in a proportion of 9 parts of blood to 1 part of anticoagulant solution.
2. Centrifuge the blood sample at 3,000 rpm for 10 minutes at room temperature.
3. Freeze the plasma at -20°C or colder. Thaw plasma at 37°C for 15 minutes.
4. Dilute plasma sample 1:100 in Sample Buffer.

ASSAY PROCEDURE

1. Remove the necessary number of precoated micro-test strips from the foil pouch and place them in the plate holder. Tightly reseal the foil pouch with the desiccant inside and store at +2°C - +8°C.

2. Add 100 µL of vWF standard or diluted sample to micro-test wells, cover with lid and incubate for 1 hour at room temperature. Perform measurements in duplicate.

3. Wash wells 4 times with wash buffer (250 µL per well).

4. For each micro-test strip used, add 20 µL of HRP-conjugated detection antibody to 2 mL of Detection Antibody Diluent. Add 100 µL of diluted detection antibody to each well, cover with the lid and incubate for 1 hour at room temperature.

5. Wash wells 4 times with wash buffer (250 µL per well).

6. Add 100 µL of substrate solution to each well, cover with lid and incubate for 20 minutes at room temperature. A blue color will develop.

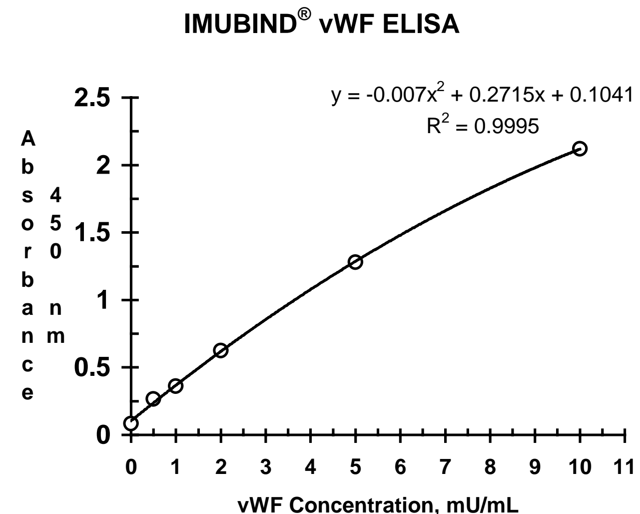
7. Stop the enzymatic reaction by adding 50 µL of 0.5M H_2SO_4. Tap the sides of the strip-wells to ensure even distribution of the H_2SO_4. The solution color will turn yellow. Read the absorbances of the solutions on a micro-test plate reader at a wavelength of 450 nm immediately. Deduct the background average of the "0" standard from the absorbances of the remaining standards and the sample readings.

CALCULATION OF RESULTS

Use the mean absorbance value for each diluted sample to interpolate its vWF concentration from the standard curve. Multiply the concentration determined from the standard curve by 100 (the dilution factor) to obtain the vWF concentration in the original plasma sample.

REPRESENTATIVE STANDARD CURVE

The standard curve is constructed by plotting the mean absorbance value calculated for each vWF standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed.



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