vW Select™ 15

(Ristocetin Cofactor Assay)
Catalog Number 107056

PRODUCT DESCRIPTION

vW Select is a performance optimized Ristocetin Cofactor Assay. Reagents, platelets, reference and control plasmas have been selected based on their combined performance and repeatability. Lot specific technical instructions are provided to assure improved performance compared to assays with random combinations of test components. ***USERS SHOULD READ AND FAMILIARIZE THEMSELVES WITH THE PROCEDURES IN THIS TECHNICAL BULLETIN BEFORE USING THIS PRODUCT.***

INTENDED USE

vW Select is used for the determination of ristocetin cofactor activity in citrated plasma. ***FOR PROFESSIONAL USE ONLY.***

PRINCIPLE

Ristocetin Cofactor Activity is the in-vitro measurement of von Willebrand Factor Function. Ristocetin causes the agglutination of platelets in the presence of vWF.[12-14] Decreased von Willebrand Factor is associated with von Willebrand Disease. The quantitation of Ristocetin Cofactor Activity is useful in the evaluation and diagnosis of this coagulopathy and when monitoring patient response to therapy.[11,13-15,18,19] Levels of ristocetin cofactor activity are determined by the ability of a test plasma and ristocetin to induce the agglutination of a standardized platelet suspension.[16] Results are determined using a lot specific Standard Reference Curve.

PRECAUTIONS

vW Select components are for ***IN-VITRO DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION***. The plasma and platelets have been tested at the source and found to be negative for HIV-1Ag, anti-HIV-1/2, Hepatitis B surface antigen, Hepatitis C antibody, Human T-Lymphotropic Type I and II (anti-HTLV I/II) and negative by a serological test for Syphilis. All materials of human origin are potentially hazardous. Observe standard precautions.[2]

THE SUBSTITUTION FOR OR USE OF ASSAY MATERIALS, OTHER THAN THOSE BIO/DATA CORPORATION PRODUCTS SUPPLIED HEREIN WILL MITIGATE TEST PERFORMANCE, ACCURACY AND PRECISION OF TEST RESULTS.

MATERIALS PROVIDED

The following reagents are provided in the kit and should be stored at 2° to 8°C prior to use:

1. Lyophilized Platelets, 1 x 6.0 mL vial
2. vW Ristocetin Reagent (Ristocetin sulfate), 1 x 0.8 mL vial @ 10 mg /mL
3. Diluent, Purified Water, 3 x 1.0 mL vials
4. Normal Reference Plasma (von Willebrand Factor), 1 x 0.5 mL vial, standardized to 90-110% von Willebrand Factor activity using World Health Organization traceable reference material
5. Abnormal Control Plasma (von Willebrand Factor Deficient), 1 x 0.5 mL vial
6. Normal Control Plasma (von Willebrand Factor), 1 x 0.5 mL vial
7. TRIS Buffered Saline, pH 7.5, 1 x 10.0 mL vial

MATERIALS REQUIRED BUT NOT PROVIDED

1. Light Transmission (platelet) Aggregometer (LTA)
2. Electronic or manual pipettor and pipette tips
3. Disposable stir bars
4. Disposable aggregometer test tubes (siliconized)
5. Mechanical rotation (rocker) device (Do not use vortex mixer)
6. Polypropylene capped sample tubes or siliconized glass tubes, and electronic or plastic transfer pipettes
7. Platelet Function Centrifuge PDQ® or general purpose laboratory centrifuge
8. Sodium Citrate 0.11M

INSTRUMENTATION

The information and instructions provided are for light transmission aggregometers using a standard test volume of 500 µL (400 µL platelet suspension, 50 µL ristocetin solution and 50 µL sample) or a micro test volume of 250 µL (200 µL platelet suspension, 25 µL ristocetin solution and 25 µL sample).

vW Select will perform as described when used on most Light Transmission (platelet) Aggregometers (LTA).[1,6,7] Follow the manufacturer's instructions for operating the aggregometer in use. Expected results generated by platelet aggregometers may vary from those cited in this Technical Bulletin.

SPECIMEN COLLECTION AND TEST SAMPLE PREPARATION

1. PATIENT PREPARATION:

Patients should fast and avoid fatty foods and dairy products for 12 hours prior to specimen collection.[6,7]

2. SPECIMEN COLLECTION:

Blood collection should be performed with care to avoid stasis, hemolysis and contamination by tissue fluids or exposure to glass.[20] Keep specimens at room temperature.[8]

Inaccurate test results might be obtained from specimens that are hemolytic, RBC contaminated, lipemic, chylous, icteric, clotted or hypofibrinogenemic and should be rejected.

Reuse of disposable items may result in inaccurate test results.

Observe standard precautions throughout the specimen collection, sample preparation, sample handling and analytical processes. [2, 3] Dispose of sharps and biological waste in accordance with laboratory policy.

Evacuated Collection Tube or Syringe Technique:

1. Draw blood using a 21G x ¾ winged needle set (BD Vacutainer® #67251 or Greiner Bio-one Vacuette® #450085) connected to a tube holder or plastic 10 mL syringe.
2. For evacuated collection tubes, use 3.5 mL tube(s) containing 3.2% sodium citrate.
3. For syringe technique, using a 10mL syringe, add 9.0 mL of freshly drawn blood into a 15 mL capped plastic polypropylene centrifuge tube(s) containing 1.0 mL of 0.11 M sodium citrate.
4. Recap tubes and immediately invert tubes 4-5 times to gently mix.

NOTE: When using evacuated collection tubes verify that the anticoagulant is 3.2% sodium citrate by checking the label. (Tube top color coding does not vary with differing citrate concentrations).

PREPARATION OF PLATELET FREE PLASMA

Platelet Function Centrifuge, PDQ (4440 x g**)**

1. Centrifuge blood using the PFP mode (180 second spin) to produce the patient test plasma.[21,22,25]
2. Remove plasma from cells, being careful not to disturb the buffy coat. Plasma should be free of red cells and platelets.
3. If testing is delayed, refrigerate the separated plasma at 2° to 8°C for a maximum of 4 hours. (If longer than 4 hours see preparation of PFP for frozen samples.) [7]

General Purpose Laboratory Centrifuge

1. Centrifuge blood at 2500 x g for 20 minutes.
2. Remove plasma from cells, being careful not to disturb the buffy coat. Plasma should be free of red cells and platelets.
3. If testing is delayed, refrigerate the separated plasma at 2° to 8°C for a maximum of 4 hours. (If longer than 4 hours see preparation of PFP for frozen samples.) [7]

Refer to the current Clinical Laboratory Standards Institute® (CLSI) Guidelines for Platelet Function Testing by Aggregometry, H58 A and Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity, H51 A[6, 7] and Determination of Factor Coagulant Activity, H48 A.[24]

NOTE: TO OBTAIN ACCURATE TEST RESULTS ASSAYS FOR VON WILLEBRAND FACTOR ACTIVITY SHOULD BE RUN ON PLATELET FREE PLASMA.[7] ONLY PLATELET FREE PLASMA SAMPLES SHOULD BE FROZEN FOR LATER VWF ANALYSIS.[2]

NOTE: Preanalytical practices are a primary source of assay imprecision. Specimen collection and quality, transport mechanics and specimen storage conditions as well as standardized sample preparation are critical requirements for obtaining accurate and reproducible test results.[18]

PREPARATION OF PLATELET FREE PLASMA SAMPLES FOR FROZEN STORAGE

Because platelets contain about 20% of vWF, it is essential to use Platelet Free Plasma for frozen transport or long term storage. A two step process

is required when a general purpose laboratory centrifuge is used to prepare Platelet Free Plasma. Centrifuge the primary specimen collection tube at 2500 x g for 20 minutes to produce Platelet Poor Plasma.Transfer the PPP to a plastic sample tube and cap. Centrifuge the PPP again at 2500 x g for 20 minutes. Transfer the supernatant to a clean plastic sample tube and cap prior to freezing. Samples can be stored at ≤ -20° C for 2-4 weeks. For longer storage periods temperatures ≤ -70°C are recommended.[7,20,22,26,27]

Note: Samples should not be frozen in an ordinary household freezer nor stored in a self-defrosting freezer because the continuous freeze-thaw cycle adversely affects specimen integrity.

INSTRUCTIONS FOR PREPARING THE vW SELECT COMPONENTS:
The following are lot specific instructions for this Kit.

The vW Select C/N 107056 is a specific combination of components for use in performing the ristocetin cofactor assay. vW Select components are optimized and tested as a system for optimum assay performance. The vW Select contains the following components and specific lot combinations. This combination has been tested and found to conform to improved expectations.

NOTE: All kit components must be at room temperature (15° to 28°C) before reconstitution.

RECONSTITUTION
LYOPHILIZED PLATELETS (1 x 6.0 mL vial)
Lyophilized Platelets: Reconstitute with 6.0 mL of TRIS buffered saline. Allow the material to rehydrate for 5 minutes. Place on rocker and rock no more than 20 minutes. If reconstituted platelets have been refrigerated it is necessary to rock the platelets for 20 minutes to allow the platelets to equilibrate and de-gas. The reconstituted platelet material should be maintained at room temperature (15° to 28°C) while being used for testing. When testing is complete any remaining reconstituted platelets can be stored refrigerated (2° to 8°C) for up to 30 days.

vW NORMAL REFERENCE PLASMA (1 x 0.5 mL vial)
vW Normal Reference Plasma: Reconstitute with 0.5 mL of purified water. Allow the material to rehydrate for 10 minutes. Invert to mix. Allow an additional 5 minutes rehydration. Invert for the final mixing. The material is now ready for use and is stable for 4 hours when refrigerated at 2° to 8° C in its original closed container. Normal Reference Plasma dilutions are stable for 40 minutes once diluted.

vW ABNORMAL CONTROL PLASMA (1 x 0.5 mL vial)
vW Abnormal Control Plasma: Reconstitute with 0.5 mL purified water. Allow the material to rehydrate for 20 minutes at room temperature. Invert to mix. Reconstituted plasma is stable for 8 hours when stored in the original closed container at 2° to 8°C. Control plasma is stable for 45 minutes at room temperature once diluted. The ability to recover specific values in the low range of the assay is dependent upon the quality of the curve that is constructed. Users should establish their own specific ranges based on the accepted curve.

vW NORMAL CONTROL PLASMA (1 x 0.5 mL vial)
vW Normal Control Plasma: Reconstitute with 0.5 mL purified water. Allow the material to rehydrate for 20 minutes at room temperature. Invert to mix. Reconstituted plasma is stable for 8 hours when stored in the original closed container at 2° to 8°C. Control plasma is stable 45 minutes at room temperature once diluted.

vW SELECT RISTOCETIN REAGENT (1 x 0.8 mL @ 10 mg/mL vial)
vW Select Ristocetin: Reconstitute with 0.8mL purified water, gently invert vial to mix and allow to rehydrate for 30 minutes at room temperature. Mix material prior to transferring the material to the disposable aggregometer test tubes. The reconstituted vW Select Ristocetin material should be maintained at room temperature (15° to 28°C) while being used for testing. When testing is complete the material may be stored refrigerated (2° to 8°C) for up to 7 days. The reconstituted vW Select Ristocetin may be aliquoted and stored frozen (-35° to -70°C) for up to 30 days. Frozen material should be thawed at 37°C, mixed and equilibrated to room temperature prior to use.

TEST PROCEDURE
The vW Select will perform as described when used with most Light Transmission (platelet) Aggregometers[1,6,7]. Follow the manufacturer's instructions for operating the aggregometer.

A. Preparation of the Blank
Platelet aggregometers each have specific test volume requirements. For improved performance to be achieved, a specific ratio of lyophilized platelets and TRIS buffered saline (TBS) will be used to prepare the blank. For this lot of vW Select, use the following amount of lyophilized platelets and TRIS buffered saline (TBS).

* Aggregometers using a STANDARD test volume of 500 µL:
350 µL Lyophilized platelets +XXX µL TBS

* Aggregometers using a MICRO test volume of 250 µL:
175 µL Lyophilized platelets + XXX µL TBS

Add a stir bar to the aggregometer test tube and then add the Lyophilized Platelets/TBS mixture. Seal the tube with Parafilm® or similar material. If stirring is not available, mix sample well by inverting several times so that the suspension will be homogeneous. The blank is stable for four (4) hours and should be prepared fresh after that time.

B. Preparation of the Standard Reference Curve
1. Prepare the following normal reference plasma dilutions for the standard curve in polypropylene tubes. Label for each dilution.
2. Dilutions should be made in an absolute manner. Serial dilutions should not be made.
3. ***Always add the TBS to the normal reference plasma.***

% Activity (dilution)	Volume of Normal Reference Plasma	Volume of TBS (diluent)	Total Volume	Instructions
100 % (1:2)	200 µL	200 µL	400 µL	Add TBS to normal reference plasma as indicated. Mix gently. Allow to stand for 10 minutes. Use within 40 minutes of preparation.
50 % (1:4)	100 µL	300 µL	400 µL	Add TBS to normal reference plasma as indicated. Mix gently. Allow to stand for 10 minutes. Use within 40 minutes of preparation.
25 % (1:8)	50 µL	350 µL	400 µL	Add TBS to normal reference plasma as indicated. Mix gently. Allow to stand for 10 minutes. Use within 40 minutes of preparation.
12.5 % (1:16) Optional	25 µL	375 µL	400 µL	Add TBS to normal reference plasma as indicated. Mix gently. Allow to stand for 10 minutes. Use within 40 minutes of preparation.

C. Preparation of the Test Plasma Dilutions
1. Label a tube (sample identification) for each sample to be tested (normal control plasma, abnormal control plasma and patient plasma).
2. Prepare a 1:2 dilution for each sample. Pipette 0.1 mL (100 µL) of test sample and 0.1 mL (100 µL) TRIS buffered saline into a polypropylene or glass siliconized tube. Mix tubes well.
3. Always add the TBS to the plasma.

D. Performing the assay

	General Instructions	STANDARD test volume 500µL	MICRO test volume 250µL
1	Pre-incubate test tubes at 37°C with stir bar	Pre-incubate test tubes in the incubation wells and **add stir bar.** Incubate one minute	Pre-incubate test tubes in the incubation wells and **add stir bar.** Incubate one minute
2	Set blank on instrument using prepared **BLANK**	**BLANK**	**BLANK**
3	Add ristocetin to test tube	50 µL	25 µL
4	Add platelet suspension to test tube	400 µL	200 µL

5	Move test tubes to test wells	Move test tubes to test wells; allow to stir for **2 minutes**	Move test tubes to test wells; allow to stir for **2 minutes**
6	Start test Channel, observe for stable baseline	Press **START** Button	Press **START** Button
7	When baseline is stable, add reference, control or patient test plasma	Add 50 µL test plasma	Add 25 µL test plasma
8	Allow aggregation pattern to develop, **STOP** when test is complete	Allow aggregation pattern to develop, **STOP** when test is completed	Allow aggregation pattern to develop, **STOP** when test is completed
9	Repeat above for each additional test or channel	Repeat above for each additional test or channel	Repeat above for each additional test or channel

EXPECTED SLOPE VALUES

The expected slope values (from the standard reference curve) for this kit are:

% Dilutions	Expected Slope values
100 %	XX-XX
50 %	XX-XX

Slope values will vary due to a number of reasons such as pipetting techniques, dilution accuracy and instrument calibration. [5] The values shown above are values obtained by Bio/Data Corporation when using the PAP-4 and PAP-8E Aggregometers and only serves as a guide to indicate that your test system is within an acceptable range and the kit is working properly. As with all procedures, each laboratory should establish its own reference ranges.

QUALITY CONTROL

A von Willebrand Factor deficient plasma is included as an abnormal control and should be assayed as test plasma with an expected result of ≤ 45% activity. This control ensures that the assay is specific for the von Willebrand Factor and that agglutination will not be influenced by other normal plasma proteins. Additionally, it is suggested that both the normal and abnormal plasma controls supplied be used to verify standard reference curve acceptability for each assay run.

EXPECTED VALUES

A result of less than the laboratory's established normal reference range for von Willebrand Factor is considered abnormal and suggestive of von Willebrand Disease.[7] However, other properties of the von Willebrand molecule must be considered for diagnosis of the variant forms of von Willebrand Disease. Since reference ranges for von Willebrand Factor are dependent on blood type, each laboratory should establish blood type specific reference ranges for its patient population.[17,23]
Abnormal control plasma will yield von Willebrand Factor assay results of ≤ 45%. Laboratories should establish their own expected ranges for this material. Normal control plasma will yield von Willebrand Factor assay results of 80% to 140% activity. Laboratories should establish their own expected ranges for this material as it is diluted.

NOTE: The 2007 National Heart Lung and Blood Institute (NHLBI) guidelines for the Diagnosis, Evaluation and Management of von Willebrand Disease set the normal/abnormal breakpoint at 30% activity.[19]

LIMITATIONS

The determination of von Willebrand Factor Activity is considered by some to be the single most important assay for the determination of von Willebrand Disease. Diagnosis of the variant forms of this coagulopathy necessitates a series of clinical and laboratory evaluations including patient and family history, factor VIII related antigen, factor VIII coagulant activity and multimeric studies. [3,4,9,10] Serial assays two or more weeks apart may be required to confirm vWD diagnosis.[19]

PERFORMANCE CHARACTERISTICS

The components of vW Select were tested on the plasmas of diagnosed von Willebrand Diseased patients as well as normal patients. Studies have shown that the accuracy and sensitivity of these components were such that varying levels of von Willebrand Factor were detected. The substitution of any assay materials other than Bio/Data Corporation products supplied herein will mitigate test performance and the accuracy and precision of the test results.

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vW Select C/N 107056

LOT 34900XXX XXXX-XX

LYOPHILIZED PLATELETS
(1 x 6.0 mL vial)

LOT 35100XXX XXXX-XX

TRIS BUFFERED SALINE
(1 x 10.0 mL vial)

LOT 34100XXX XXXX-XX

vW SELECT RISTOCETIN REAGENT
(Ristocetin sulfate) 1 x 0.8 mL @ 10.0 mg/mL, vial

LOT 35200XXX XXXX-XX

vW NORMAL REFERENCE PLASMA
(1 x 0.5 mL vial)

33800XXX XXXX-XX

REFERENCE RANGE
0.[illegible]y)
90 - 110%

ASSAY VALUE
XXX

vW ABNORMAL CONTROL PLASMA
(1 x 0.5 mL vial)

LOT 33900XXX XXXX-XX

33900XXX XXXX-XX

REFERENCE RANGE
<45% Activity
<45%

ASSAY VALUE
XXX

vW NORMAL CONTROL PLASMA
(1 x 0.5 mL vial)

LOT 34000XXX XXXX-XX

XXXX-XX

REFERENCE RANGE
80 - 140% Activity
80 - 140%

ASSAY VALUE
XXX

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