vW Select™
(Ristocetin Cofactor Assay System)

PRODUCT DESCRIPTION
vW Select is a ristocetin cofactor assay system comprised of optimized reagents, control plasmas and lot specific technical information for use in the detection of von Willebrand Disease (vWD). The materials provided in the vW Select system have been specifically selected to provide improved performance and repeatability compared to ordinary component combinations found in existing ristocetin cofactor assay systems.

INTENDED USE
The vW Select Ristocetin Cofactor Assay System is optimized to elicit the agglutination of lyophilized platelets in the presence of the patient's Platelet Poor Plasma and Ristocetin.

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

PRECAUTIONS
vW Select Ristocetin cofactor Assay System components are for PROFESSIONAL LABORATORY USE ONLY AND *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. Follow standard precautions.

THE USE OF COMPONENTS OTHER THAN THOSE SUPPLIED WITH THIS KIT WILL AFFECT THE ACCURACY AND PRECISION OF TEST RESULTS.

MATERIALS PROVIDED
Store all materials at 2° - 8° C prior to reconstitution.
1. Lyophilized Platelets, 2 x 10.0mL.
2. AggRecetin®, ristocetin sulfate 2 x 15mg
3. AggRecetin Diluent, 2 x 2.0mL
4. Normal Reference Plasma (von Willebrand Factor), 5 x 0.5mL standardized to 90-110% von Willebrand Factor activity uses a World Health Organization traceable reference material.
5. Abnormal Control Plasma (von Willebrand Factor Deficient), 5 x 0.5mL.
7. Normal Control Plasma (von Willebrand Factor), 5 x 0.5mL
6. TRIS Buffered Saline, pH 7.5, 3 x 10.0mL.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified water (distilled, deionized or reagent grade), pH 5.3 - 7.2
3. Pipettors
4. Disposable Stir bars
5. Aggregometer cuvettes
6. Rocker (Mechanical Rotation Device) (Do not use vortex mixer)

INSTRUMENTATION
vW Select system will perform as described when used on most light transmission platelet aggregometers[1] (LTA). Follow the manufacturer's instructions for operating the aggregometer.

SPECIMEN COLLECTION AND PREPARATION OF TEST SAMPLE
Refer to the current CLSI Guidelines for Platelet Function Testing by Aggregometry, H58P and Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity, H51A[6,7]

1. **PATIENT PREPARATION:**
Patients should fast and avoid fatty foods and dairy products for 12 hours prior to specimen collection.[6]

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

Each of the following can cause test results to be inaccurate; and affected specimens should be rejected: hemolysis, RBC contamination, lipemia, chylous, icterus, clots in specimen, and hypofibrinogenemia. Reuse of disposable items may result in inaccurate test results.

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

Evacuated Collection Tube Technique:
1. Use a winged needle for venipuncture
2. Draw blood using plastic or siliconized tubes containing 0.11M Sodium Citrate anticoagulant
3. Gently invert specimen 4-5 times to mix.

NOTE: When using evacuated collection tubes, make sure the citrate anticoagulant is 0.11M by checking the label. Colored tops do not vary with differing citrate concentrations. Follow the manufacturer's instructions for specimen collection.

PREPARATION OF PLATELET POOR PLASMA
Platelet Function Centrifuge, Model PDQ™ and Standard Method

Model PDQ
1. Use the PFP Mode to produce the patient plasma test sample. Follow manufacturer's instructions.

Standard Method
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° - 8° C for a maximum of 2 hours.

INSTRUCTIONS FOR THE vW SELECT KIT COMPONENTS:
The following are lot specific instructions for vW Select Kit

LOT _____ ⌛ _____

The vW Select system; C/N 106730 is a specific combination of the standard components for use in performing the Ristocetin Cofactor Assay. Unique to the vW Select System configuration are specific instructions on how to achieve improved performance when using the kit. The Select Kit contains the following materials and specific lot combinations. This combination has been tested and found to conform to improved expectations. Do not change or substitute any of the materials from the lot(s) specified below. For improved performance, follow these instructions when reconstituting and performing the assay:

RECONSTITUTION
NOTE: Reagents must be at room temperature (15° - 28°C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.

LYOPHILIZED PLATELETS (2 x 10.0 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

Re-suspension of Lyophilized Platelets: To a vial of Lyophilized Platelets, add 10.0mL of TRIS Buffered Saline. Allow to rock for no more than 30 minutes. After refrigeration and prior to use, it is also necessary to rock for 30 minutes at ambient temperature to allow the reagent to equilibrate and de-gas. After reconstitution, it is recommended that the material be mixed prior to its transfer to the test tubes. The reconstituted platelet material should be maintained at room temperature (20° - 28°C) while being used for testing. When testing is complete, any remaining material may be stored refrigerated (2 - 8°C) for up to 30 days. When using the lyophilized platelet material in the vW Select system the following should be considered:

1. Although slight and not significant for standard assays, the reconstituted platelet suspension will change in activity with storage. Care should be exercised to check the assay system with control material to ascertain if an objectionable change to the platelet activity has occurred.
2. Improved results can be obtained by storing the reconstituted reagent material on the PAP-8E with stirring in the reagent wells. This will affect the stability and activity of the reagent. The lyophilized platelets may be stored at 37°C with stirring and used for up to 6 hours. Material stored at 37°C with stirring for more than 6 hours should be discarded. Contact Bio/Data Corporation or your authorized distributor for instructions in this alternate methodology and the setup of the instrument for stirring in the reagent wells.

vW NORMAL REFERENCE PLASMA (5 x 0.5 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

1. vW Normal Reference Plasma: Reconstitute with 0.5 mL of purified water. Allow the material to re-hydrate for 10 minutes. Then, invert to incorporate all of the material in the vial. Allow an additional 5 minutes re-hydration. Invert for the final mixing. The material is now ready for use and is stable for 4 hours when refrigerated at 2° - 8° C in its original sealed container.

vW ABNORMAL CONTROL PLASMA (5 x 0.5 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

Abnormal Control Plasma: Reconstitute with 0.5mL purified water. Re-hydrate 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°- 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. If assay value is reported at lower than the instruments reportable range, rerun the material undiluted. Assay value will be 1/2 of the reported undiluted value. Users should establish their own specific ranges based on the accepted curve.

vW NORMAL CONTROL PLASMA (5 x 0.5 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

Normal Control Plasma: Reconstitute with 0.5mL purified water. Re-hydrate 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°- 8°C. Control Plasma is stable 45 minutes at room temperature once diluted.

Different dilutions (1:2 or 1:4) may present different relative recoverable activity. Each dilution should have its own reference range and that established range should be used to control the assay system.

TRIS BUFFERED SALINE (3 x 10.0 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

AGGRECETIN DILUENT (2 x 2.0 mL vials) PART NUMBER: _____
LOT _____ ⌛ _____

vW SELECT AGGRECETIN, Ristocetin sulfate (2 x 15 mg vials) PART NUMBER: _____
LOT _____ ⌛ _____

* Vial Reconstitution Volume - See below

**Reconstitution of the AggRecetin*
Bio/Data Corporation has tested the above lot of vW Select AggRecetin and has determined that an improved performance will be achieved when a vial is reconstituted with:

***_____ mLs of the provided AggRecetin Diluent**

After reconstitution, invert gently to mix and allow to re-hydrate for 30 minutes at room temperature. Mix prior to transfer of the material to the test tubes. The reconstituted vW Select AggRecetin material should be maintained at room temperature (20° 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 AggRecetin 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]. Follow the manufacturer's instructions for operating the aggregometer.

The Platelet Aggregation Profiler, PAP-8E uses micro-volumes. If using a Platelet Aggregation Profiler, PAP-4, the volumes of samples and reagents should be doubled.

A. Preparation of the Blank

For improved performance to be achieved, a specific ratio of Lyophilized Platelets (LP) and TRIS Buffered Saline (TBS) will be used to prepare the blank. This ratio is based on a fixed volume of Lyophilized Platelets (LP) 175µL and a variable volume of TRIS Buffered Saline (TBS). Volume ratios may be adjusted for the aggregometer.

The TBS Adjustment Factor * provided below is multiplied by the fixed volume of Lyophilized Platelets (LP) 175µL to determine the amount of TRIS Buffered Saline (TBS) to be used to prepare the blank.

EXAMPLE:
If the TBS Adjustment Factor is 0.9, then the amount of TBS to be added to the fixed volume of Lyophilized Platelets (LP) 175µL will be 158µL.

0.9 x 175µL = 157.5µL (158µL)

* _____ Blank TBS Adjustment Factor

Add a stir bar to a test tube and then add the LP/TBS mixture to the tube. Seal the tube with Parafilm® or similar material.

When using the Platelet Aggregation Profiler, PAP-8E, the prepared blank is stored on the unit with stirring. The prepared, stirred and incubated blank is stable for four (4) hours. At the end of four hours a fresh blank should be prepared.

B. Preparation of the Normal Reference Plasma (NRP)

1. Prepare the following Normal Reference Plasma (NRP) dilutions for the standard curve. Label a tube for each dilution. See table 1.
2. Dilutions should be made in an absolute manner. Serial dilutions should not be made.
3. Always add the TBS to the NRP.

vW Normal Reference Plasma Quick Dilution Table 1:

Dilutions	Volume from Vial of NRP	Volume of TBS
1:2 (100%)	200	200
1:4 (50%)	100	300
1:8 (25%)	50	350
1:16 (12.5%) optional	25	375

4. Dilutions have limited stability. After dilutions are made, they should be inverted and then allowed to stand for 10 minutes. Invert dilutions prior to use. Dilutions are stable for up to 40 minutes after preparation.

C. Preparation of the Test Plasma Dilutions

1. Label a tube (sample identification) for each sample to be tested.
2. Prepare a 1:2 dilution for each sample. Pipette 0.1mL of test sample and 0.1mL TRIS buffered saline into the tube. Mix thoroughly by inversion.

D. Performing the Assay

Performing the assay using the Platelet Aggregation Profiler, PAP-8E:

1. Place the appropriate number of cuvettes required for testing into the incubation wells. Add a new stir bar into each cuvette. Incubate the cuvettes for one minute without stirring.
2. Place the cuvettes into the stirred incubation wells.
3. Add 25µL vW Select AggRecetin into the pre-warmed cuvettes.
4. Add 200µL of the platelet suspension into each cuvette being careful to avoid splashing or the introduction of air bubbles. Do not allow the suspension to run down the side of the cuvette.
5. Select the Timer button and the count down will begin. Incubate the samples at 37ºC for 2 minutes while stirring.
6. While incubation is taking place, the blank for each test channel should be set. Set the 100% baseline by placing the blank into the first test well.
   Select Blank
   The status will change to Start. Repeat for each channel.
7. Place the cuvettes of vW Select AggRecetin/platelet suspension into the test wells. Close the well cover.
   Select Start, channel 1
   Before the sample is added, % aggregation should stabilize to a constant between ± 3% aggregation. If this does not happen, the blank was not set correctly or the incubation cycle was faulty.
8. When the baseline is stable, add 25µL of the 100% dilution of the normal reference plasma to the cuvette in channel one.
   Select Inject
   Observe that the instrument has started to measure and record the % aggregation. You should observe that the baseline % aggregation will change when the sample is added but will reset to 0% aggregation after the "Inject" button is pressed. Do not allow plasma dilution to run down the side of the cuvette. Be careful not to spike the platelet suspension with pipetting technique. The select button will change color from green to grey. A point will appear on the graph to indicate the time the inject selection was made.
9. Repeat Step 8 for the 50%, channel 2 and 25%, channel 3, normal reference plasma dilutions, substituting each of these dilutions for the 100% normal reference plasma dilution.
10. For the test sample repeat steps 1-8, substituting the test plasma for reference plasma dilution in step 8.
11. The test will run for 6 minutes.
12. When all tests are complete the PAP-8E will calculate the percent activity for each test. Selecting the Curve button, the results will be displayed on the standard curve. The printout will include the aggregation patterns, standard curve and the calculated results.

QUALITY CONTROL

A von Willebrand factor deficient plasma is included as an abnormal control and should be assayed as a test plasma with an expected result of ≤ 45% activity. This control ensures that the assay system 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 run to validate standard curves.

EXPECTED VALUES

A result of less than the laboratories established normal reference range for von Willebrand factor is considered abnormal and suggestive of von Willebrand Syndrome.[7] However, other properties of the von Willebrand molecule must be considered for diagnosis of the variant forms of von Willebrand Syndrome. 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]

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.

LIMITATIONS

The determination of von Willebrand factor is considered by some to be the single most important assay for the determination of von Willebrand Syndrome. 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[3,4,9,10] and multimeric studies.[9,10] Serial assays may be required to confirm diagnosis.

PERFORMANCE CHARACTERISTICS

The components of vW Select system were tested on the plasmas of diagnosed von Willebrand Syndrome 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 use of components not supplied with the kit will affect the accuracy and precision of test results.

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NOTE: Also available, **but cannot be substituted or used to replace the components supplied in the vW Select Kit.**

For a complete list of available products please go to our web site www.biodatacorp.com or contact customer service below.

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