AggRecetin®
(Ristocetin A Sulfate)

PRODUCT DESCRIPTION
AggRecetin® is lyophilized Ristocetin A Sulfate, a substance of unknown chemical structure which is isolated from *Nocardia lurida*. AggRecetin contains in excess of 90% Ristocetin A.

INTENDED USE
AggRecetin reagent is the required reagent, used in combination with other IVD components, to perform a Ristocetin Cofactor Activity test on Platelet Rich Plasma.

PRINCIPLE
In the presence of the von Willebrand factor, AggRecetin induces agglutination of platelets in platelet rich plasma or standardized fixed platelet suspensions.[11-13]

PRECAUTIONS
AggRecetin is for PROFESSIONAL LABORATORY USE ONLY AND *IN-VITRO* DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.

NOTE TO USER: Any serious incident that occurs in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS PROVIDED
AggRecetin is provided in standard and variable final (in test) concentrations. (See reconstitution.) Store at room temperature prior to reconstitution.

1. 1.0-1.5 mg/mL 15 mg AggRecetin and 2.0mL diluent (0.85% Saline)
2. 100 mg Bulk AggRecetin only

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Pipettors (0.5mL, 0.45mL, 0.5mL volumes)
3. Disposable Stir Bars
4. Aggregometer cuvettes

INSTRUMENTATION
AggRecetin will perform as described when used on most optical platelet aggregometers[1]. Follow the manufacturer's instructions for operating the aggregometer in use.

SPECIMEN COLLECTION AND PREPARATION OF TEST SAMPLE

Refer to the current NCCLS Approved Guideline H21 A2 for detailed specimen collection and sample preparation instructions.[6]

1. **PATIENT PREPARATION:**
   Patients should refrain from taking aspirin or medications containing aspirin, other medications and dietary supplements known to affect platelet function for 7 - 10 days prior to specimen collection. 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, thrombocytopenia (<75,000/mm^3) 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.

   Syringe Technique (recommended)[8]
   a. Use a butterfly needle for the venipuncture
   b. Draw 9.0mL of blood into a plastic syringe. Avoid excess suction.
   c. Remove the needle from the syringe and immediately and gently dispense the blood into a plastic [polypropylene][4] tube containing 1.0mL of 0.11M Sodium Citrate anti-coagulant. The ratio of blood to anti-coagulant must be 9 parts of blood to 1 part anti-coagulant.[5]
   d. Cover and invert 4-5 times gently to mix.
   e. Maintain at room temperature (15° to 28°C)

   NOTE: When the patient's hematocrit is < 30% or > 55%, the blood to anti-coagulant volumes must be adjusted.[4]

   Evacuated Collection Tube Technique
   1. Use a butterfly needle for the venipuncture
   2. Draw blood using (plastic) tubes containing 0.11M Sodium Citrate anti-coagulant
   3. Gently invert 4-5 times to mix

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

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

Catalog No. 100970
1. 1.0-1.5mg/mL Final/10-15mg/mL Working
   a. Refer to dilution chart below for the desired concentration of AggRecetin and required diluent volume.
   b. Add selected volume of diluent to the glass vial of AggRecetin.
   c. Invert gently to mix. Allow to stand until completely dissolved.

Catalog No. 101241 (100 mg)

1. Bulk
   a. Place 15mg of AggRecetin in a 10mL glass vial.
   b. Reconsitiute with 0.85% Saline
   c. Refer to the Dilution Chart for the desired concentration of AggRecetin and required diluent volume.
   d. Add selected volume of diluent to the vial of AggRecetin.
   e. Invert gently to mix for 30 minutes. A specimen rocker can be used.

DILUTION CHART
All Final concentrations are based upon 0.05mL of AggRecetin added to 0.45mL of platelet rich plasma.

To 15mg AggRecetin Add Diluent In The Amount Of	Working Concentration (as reconstituted)	Desired AggRecetin Final Concentration (in test)
1.00 mL	15 mg/mL	1.5 mg/mL
1.07 mL	14 mg/mL	1.4 mg/mL
1.15 mL	13 mg/mL	1.3 mg/mL
1.25 mL	12 mg/mL	1.2 mg/mL
1.36 mL	11 mg/mL	1.1 mg/mL
1.50 mL	10 mg/mL	1.0 mg/mL

REAGENT STORAGE
The reconstituted AggRecetin is stable for 7 days when stored at 2° - 8°C in its original tightly sealed container. For long term storage, freeze reconstituted AggRecetin at -20°C for up to 8 weeks. Once thawed use within 8 hours. Reagent needs to be gently inverted for 30 minutes while reaching room temperature.

TEST PROCEDURE
Testing must be completed within 3 hours of specimen collection.[8]
1. Place a stir bar in each cuvette
2. Prepare an aggregometer blank by pipetting 0.5mL platelet poor plasma into a cuvette.
3. Pipette 0.45mL platelet rich plasma into a second cuvette. Incubate at 37°C for 3 minutes.
4. Set, if required, the 0% and 100% baselines according to the manufacturer's instructions for the aggregometer in use.
5. Add 0.05mL AggRecetin directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette.
6. Allow the aggregation pattern to generate for 5 minutes.

QUALITY CONTROL
Laboratories should follow generally accepted quality control practices when assay specific proficiency testing is not available.

To assure proper instrument operation and reagent performance, a control specimen should be evaluated each day that tests are performed. The control specimen should be prepared in the same manner as the test specimen. For qualitative platelet aggregation studies, the control should consist of fresh platelet rich plasma collected from a (specified and qualified) normal donor who has not ingested aspirin containing compounds within 10 days of testing and has a history of normal platelet function.

EXPECTED VALUES

Expected ranges for each reagent at various concentrations used to induce platelet aggregation should be established by each laboratory, see Table 2.[4,8,9,10]

Table 2

TYPICAL PLATELET AGGREGATION RESPONSES FOR NORMAL DONORS @ 250,000 PLATELETS/mm³ [total aggregation at 5 minutes]

	AggRecetin (Ristocetin Sulfate)	AggRecetin
Final Conc.	1.5 mg/mL	1.0 mg/mL
Lag Phase [sec]	0	-
Primary Slope	32-63	15-34
Total Aggregation (%@5min)	68-106	55-80
Biphasic Aggregation	Maybe at lower concentrations	Variable
Other	---	Normal donors may vary

LIMITATIONS

A detailed patient history is required for accurate test interpretation. Patients should be questioned about the recent ingestion of any medication, because a number of prescription and nonprescription drugs may interfere with platelet aggregation. Substances such as caffeine, tobacco, herbal extracts (or supplements) and alcohol may affect results.[7,8]

PERFORMANCE CHARACTERISTICS

Studies have shown that this product will perform as described prior to its expiration date when procedural and storage directions are followed.

Linearity:

Platelet aggregation induced by common agonists (Ristocetin A) is a nonlinear test system for the following parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by many factors such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reactants or their concentration.

ACCURACY, PRECISION AND REPRODUCIBILITY

Accuracy

In platelet aggregation, accuracy is a relative parameter and is dependent on the test system.

Precision and Reproducibility

The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges. However, there is an experienced based consensus for these parameters (see below). Each laboratory must establish its own limits for test acceptability.

Test to Test Reproducibility:	less than ± 7.5%
Instrument to Instrument Reproducibility:	less than ± 15%
Reagent Lot to Lot Variation:	less than ± 10.5%
Laboratory to Laboratory (same test system):	less than ± 12.5%

Note: When comparing Rictocetin Cofactor to von Willebrand Factor Antigen for diagnostic interpretation, note that patients with Type O blood have significantly lower plasma levels of von Willebrand Factor Antigen than other blood types.[8]

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