IVD	In-Vitro Diagnoastic Device
REF	Catalog Number
	Single Use
	Caution
	Temerarture Limitations
NON STERILE	Non-Sterile
	Must Read
	Consult Instructions for Use
	Bio-Hazardous
UK REP	United Kingdom Rep
EC REP	European Union Rep.
UK CA	United Kingom Marked & Registered Product
CE	CE Marked & Registered Product
	Manufacturer

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BIO/DATA CORPORATION

155 Gibraltar Road, Horsham, PA 19044 U.S.A.
(800) 257-3282 U.S.A. (215) 441-4000 Worldwide
(215) 443-8820 Fax Worldwide
E-mail: customer.service@biodatacorp.com
Internet: www.biodatacorp.com
An ISO 13485 Registered Company

UK REP Alpha Laboratories Ltd, 40 Parham Drive, Eastleigh, Hampshire, SO50 4NU United Kingdom

EC REP mdi Europa GmbH, Langenhagener Str. 71, 30855 Langenhagen, GERMANY

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Instructions for Use No. 101242 Rev. T

English

AggRecetin®

IVD

(Ristocetin A Sulfate)

REF 100970 REF 101241

PRODUCT DESCRIPTION

AggRecetin is Ristocetin A Sulfate, a glycopeptide of unknown chemical structure which has been isolated from Nocardia lurida. AggRecetin contains in excess of 90% Ristocetin A. It has been optimized for use with Light Transmission Aggregometers but may also be used with other turbidometric or impedance analyzers.

INTENDED PURPOSE

Detection/Measurement

AggRecetin is used, in conjunction with other IVD products, diluents and controls, to measure Ristocetin Cofactor Activity which provides semi-qualitative information on the presence and state of von Willebrand Factor in test samples.

Product Function

AggRecetin principally functions to provide information on the presence of von Willebrand Disease or Bernard-Soulier Syndrome in people exhibiting muco-cutaneous bleeding, heavy menstrual bleeding or other clinical symptoms of a bleeding disorder. This information, when interpreted by a trained individual, is utilized alongside other information such as the patient's medical history, physical condition, as well as results from other medical and laboratory testing, to render a professional opinion on the need for further testing for a medical diagnosis.[8,9,1,11,13]

Specific Information Provided

AggRecetin is not intended for the detection or definition of a specific disorder, condition, or risk factor. It induces the agglutination of platelets in a test sample, the rate and extent of which is captured and displayed by a Light Transmission Platelet Aggregometer. The data generated by the addition of AggRecetin to a test sample, when AggRecetin is used as intended, provides trained professionals information which may define the physiological or pathological state of the test specimen.

Automation

AggRecetin is a reagent and therefore not automated, however it is intended for use in semi-automated and automated Light Transmission Platelet Aggregometers.

Quality/Quantity

There is no absolute standard for the Ristocetin Induced Platelet Aggregation or Ristocetin Cofactor Activity Assays. The World Health Organization describes the von Willebrand Factor 'standard' provided for these tests as having an estimated value. The test is referred to by various professional and standards organizations as semi-qualitative or semi-quantitative for this reason.

Specimen Type

Platelet Rich Plasma (PRP) from humans or animals is used for the Ristocetin Induced Platelet Aggregation or RIPA test. Results are based on the difference in the extent of aggregation between high and low concentrations of AggRecetin.

AggRecetin can be used with human or animal Platelet Poor Plasma (PPP) along with reference plasma, lyophilized, formalin fixed platelets, controls and diluents to perform a Ristocetin Cofactor Activity test. Test results are interpolated from a standard curve.

Testing Population

Human: the prevalence of the von Willebrand abnormality is global and may vary by race, ethnicity, blood type and other factors. The Incidence is ~2%.

Animal: Prevalence is species dependent. Incidence is species dependent.

In Vitro Diagnostic

AggRecetin is an in vitro diagnostic reagent intended for Professional Laboratory Use Only. It is not intended for injection or ingestion.

Intended User

AggRecetin is intended for use by qualified laboratory personnel.

Test Principle

When AggRecetin is added to Platelet Rich Plasma it induces passive platelet agglutination in the presence of von Willebrand Factor. The rate and extent of platelet agglutination is recorded using a Light Transmission Platelet Aggregometer.

AggRecetin can be used with fixed platelets that have the glycoprotein GPIb receptor exposed for the Ristocetin Cofactor Activity test and in the Ristocetin Induced Platelet Aggregation (RIPA) test in wich various concentrations of AggRecetin induce agglutination in platelet rich plasma.[11,12,13,15,16]

Calibrators and Controls

There are no calibrators or controls required for AggRecetin. Its use is concentration dependent. It is recommended that a known donor sample be tested with each lot of AggRectin.

Reagent Limitations

AggRecetin will perform as specified when the Instructions for Use are followed and when used prior to the expiration date printed on each vial.

Reagents Provided

REF 100970: 1 vial of AggRecetin (15.0 mg) and 1 vial of 2.0 mL of diluent.②
REF 101241: 1 vial of AggRecetin (100.0 mg)

Reagents and Materials Required But Not Provided

Reagents

REF 101241: 0.85% physiologic saline for diluent

Materials And Accessories

Platelet Aggregometer (follow the manufacturer's Instructions for Use)
Centrifuge
Electronic Pipette
Pipette Tips②
Aggregometer Test Cuvettes (siliconized)②
Stir Bars (plastic coated)②
Plastic Sample Tubes and Caps (for dilutions) ②

Note: disposable items such as cuvettes, stir bars, samples tubes and caps are for one time use only

Storage and Stability

AggRecetin does not require temperature protection for shipment
Store AggRecetin at 2 – 8 °C in its original packaging prior to use

Reconstituted AggRecetin is stable for 7 days when stored in its tightly capped, original container at 2 – 8 ° C

For long term storage, freeze the reconstituted AggRecetin at - 20° C for up to 8 weeks.

Once thawed, use within 8 hours. AggRecetin needs to be gently mixed for 30 minutes while reaching room temperature.

Sterility

NON STERILE

AggRecetin is not a sterile product. Be careful not to contaminate AggRecetin when pipetting the reconstituted or aliquoted reagent.

Warnings and Precautions

Wear PPE in accordance with laboratory policies and practices when handling AggRecetin
Follow standard precautions when preparing specimens and testing samples
Handle AggRecetin with care to avoid contamination during use
Avoid reagent evaporation by limiting air – liquid exchange surfaces
To assure optimum test results, a known donor or controls and tests should be run in succession and without interruption.
To preserve reagent stability, seal the vial with its original cap after use
Dispose of post-test materials in accordance with applicable regulations and laboratory polices.[2,5]

Note to user: any serious incident that occurs in relation to this product shall be reported to the manufacturer and the competent authority of the Member State in which the user/and or patient are established.

Infectious Material Status

AggRecetin and its diluent do not include any infectious materials. Test specimens and samples must be considered infectious and should be handled as if capable of transmitting infection and after testing be disposed of in compliance with applicable regulations and laboratory policies.

Special Facilities

AggRecetin does not require the use of special facilities within a laboratory environment

Preparation for Use

Note: AggRecetin must be at room temperature (15 – 28 ° C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.

Reconstitution

REF 100970: 15.0 mg of AggRecetin. Working concentration 15.0 mg/mL. Refer to the table below to prepare desired working and final concentrations. All final concentrations are based on adding 25 uL of AggRecetin to a 225 uL test sample.

Add the selected amount of diluent to the AggRecetin vial. Mix gently by inversion. Allow AggRecetin to stand until completely dissolved.

Dilution Table

To 15 mg AggRecetin add Diluent in the Amount of	Working Concentration (as reconstituted)	Final Concentration (in test sample)
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

Further dilutions may be made using 0.85% (physiologic) saline.

Note: Do Not use blood bank saline

REF 101241: 100 mg AggRecetin.
-Place 15 mg of AggRecetin into a 10 mL glass vial
-Reconstitute with Tris Buffered Saline (TBS) or 0.85% physiologic saline
-Cap reconstituted AggRecetin prior to use.
-Refer to the Dilution Table to determine the amount of diluent required for the desired concentration
-Invert gently to mix for 30 minutes using a specimen rocker

Patient Preparation

Patients should refrain from taking Aspirin or using Aspirin containing medications and products, and other medications, supplements or energy drinks known to affect platelet function for 7 – 10 days prior to specimen collection. Patients should refrain from ingesting fatty foods and dairy products and smoking for 12 hours before specimen collection.[6,7,14,15]

Note: Consultation with the physician prior to any medication change or avoidance is required.

Specimen Collection

The specimen should be collected with care to avoid stasis, hemolysis, contamination by tissue fluid and exposure to glass. Specimens must be kept at room temperature. Release the tourniquet as soon as blood begins to flow into the collection device.

Practice standard precautions throughout the specimen collection, sample preparation and analytical processes. Dispose of sharps and biohazardous waste in accordance with applicable regulations and laboratory policies.[2,5,14,15]

Evacuated Specimen Collection Tube Technique:
-use a 21g or 23g winged needle collection set for specimen collection
-draw blood into plastic evacuated specimen collection tubes containing 3.2% sodium citrate anticoagulant
- gently mix the specimen collection tube 4 -5 times by inversion
-note collection time on the specimen label
-maintain specimen collection tubes at room temperature
-remix specimen collection tubes prior to centrifugation

Syringe Collection Technique:
-Use a 21g or 23g winged needle collection set for the venipuncture
-draw 9.0 mL of blood into a plastic syringe, avoiding excess suction
-Clamp the winged needle tubing and disconnect the syringe
-Immediately and gently dispense the blood specimen into a plastic (polypropylene) tube containing 1.0 mL of 0.11M sodium citrate anticoagulant. The blood to anticoagulant ratio is 9 parts blood to one part anticoagulant
-Cap the plastic tube and mix 4 – 5 time gently by inversion
-Record the specimen collection time on the plastic tube label
- remix specimen collection tubes prior to centrifugation
-Maintain the specimen at room temperature

Notes:

When the patient's hematocrit is less than 30% or greater than 55%, the blood -anticoagulant ratio must be adjusted.[3,6,8]

Blue top evacuated specimen collection tubes can contain 3.2% (0.11M) or 3.9% (0.13M) sodium citrate anticoagulant. The 3.2% (0.11 M) is the recommended concentration for platelet function studies.

Sample Preparation

Platelet Rich Plasma (PRP)
-centrifuge the specimen at room temperature for 10 minutes at 150 X g
-examine the plasma
Reject the sample if there is visible hemolysis, icterus or lipemia or clots
-collect the PRP layer using a plastic transfer pipette without touching the leukocytes or red cells
-transfer the PRP to a prelabeled plastic tube and cap the tube. Maintain at room temperature

Platelet Poor Plasma (PPP)
-centrifuge the remaining specimen at room temperature for 15 minutes at 2500 X g
Reject the sample if there is visible hemolysis, icterus or lipemia or clots
-collect the PPP using a plastic transfer pipette
-transfer the PPP to a prelabeled plastic tube and cap the tube. Maintain at room temperature [1,3,6,7,8,9,10]

Assay Procedure

Note: This is a general procedure. Follow the Instructions for Use provided by the manufacturer of the aggregometer in use.

Ristocetin Induced Platelet Aggregation (RIPA):
-Prepare PRP and PPP as described in the Sample Preparation Section
-Prepare the high and low concentrations of AggRecetin to be used in the test
Common final concentrations are 1.2 or 1.0 mg/mL for high dose AggRecetin and 0.6 or 0.5 mg/mL for low dose concentrations of AggRecetin
-Set the 0 and 100% baselines using PPP and PRP according to the aggregometer manufacturer's instruction
-Pipette 225 uL of PRP into each of two test cuvettes
-Add a stir bar to the test cuvettes
-Incubate the PRP according to the manufacturer's instructions
-Pipette 25 uL of high dose AggRecetin into one cuvette and start the aggregometer recording
-Pipette 25 uL of low dose AggRecetin into the second cuvette and start the aggregometer recording
-record the agglutination responses for six minutes

Notes:
RIPA tests should be run in duplicate
Use a known donor for control samples
Each laboratory should establish and validate its own test protocol and verify the resulting performance of its test system (reagents, instrument, test protocol)[8,13,14,15]

Quality Control

For platelet aggregation studies a known donor should be tested in the same manner as the patient to assure tests system performance and consistency. A new control should be included with each test series, or at least for each new reagent lot and after instrument maintenance. Each laboratory must define its acceptable ranges and verify the expected performance of the test system.

Results

High dose AggRecetin should induce a strong, single wave in patients with no bleeding disorder. Low dose AggRecetin should yield no response in patients with no bleeding disorder. A strong response to low dose AggRecetin suggests the presence of certain types of von Willebrand Disease. In normal patients, there is little or no response to low dose AggRecetin. Results must be interpreted in light of the patient's clinical condition. A final diagnosis should not be made until further testing is completed.

Expected Values

AggRecetin interacts with vWF and the GPIb membrane receptor to cause platelet agglutination. Conventional aggregation pathways are not activated and granule release does not occur during the initial reaction. Individual responses to lower concentrations of AggRecetin can be variable.[3,4,9,16]

Typical Aggregation Responses for a Known Normal Donor @ 6 minutes (250,000plts/cumm)

	AggRecetin	AggRecetin	AggRecetin
Final Concentration	1.5 mg/mL	1.0 mg/mL	0.5 mg/mL
Lag Phase (Secs)	0	variable	-
Primary Slope	32 - 63	15 - 34	>10
Final Aggregation (% @ 6 minutes)	68 - 106	55 - 80	0 - 10
Biphasic Aggregation	No	variable	no
Other	--	Normal Donors May Vary	Normal Donors May Vary

Limitations

In Light Transmission Aggregometry, the presence of red blood cells in the PRP will cause the observed aggregation to be reduced. The presence of platelets in the PPP will cause final aggregation to be increased. Spurious results may occur if the PRP platelet count is less than 75,000 platelets/cumm. PRP platelet counts can only be performed using the hemocytometer method. Compromised samples must be rejected.

If the results are abnormal, the test should be repeated on another occasion

Each laboratory must establish reference ranges for the population it serves and the AggRectin concentrations used[7,8,11,15]

Analytical Performance

Platelet aggregation, induced by commonly used agonists like ristocetin, is a non-linear test system. Responses are based on the difference between the patient's Platelet Rich Plasma and Platelet Poor Plasma light transmission and, therefore, results are unique to that patient. Certain parameters are more prone to non-linearity than others. These include 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 the response rate or activity and is not a quantitative measure of the reactants or their concentrations.[8,9,10,11,15]

In platelet aggregation, accuracy is a relative parameter and dependent on the test system. The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges.

The nature of the Ristocetin based test systems and variability of the linearity, precision and reproducibility of the results is recognized by multiple standards organizations and in other published voluntary consensus standards. The commonly accepted CV is +/- 15% (Clinical and Laboratory Standards Institute (among others), Platelet Function Testing by Aggregometry, Approved Guideline, H 58-A). WHO and its laboratory partner, the National Institute for Biological Controls and Standards, provide estimates for von Willebrand Factor measurements.

Test to Test Reproducibility:	less than +/- 7.5%
Instrument to Instrument Reproducibility:	less than +/- 15.0 %
Reagent Lot to Lot Variability:	less than +/- 10.5%
Laboratory to Laboratory: (same test system)	less than +/- 12.5%

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