



Ristocetin
REF AG004K
R1 3 x 7.5 mg



www.hyphen-biomed.com

155, rue d'Eragny
 95000 NEUVILLE SUR OISE
 FRANCE
 Tel. : +33 (0)1 34 40 65 10
 Fax : +33 (0)1 34 48 72 36
 info@hyphen-biomed.com

Ristocetin for platelet aggregation tests

Not for Sale in the US

English, last revision: 08-2017

INTENDED USE:

For *in vitro* diagnostic use. Measurement of platelet aggregation.

SUMMARY AND EXPLANATION:

Ristocetin reagent is available for use in Ristocetin-induced platelet aggregation (RIPA) tests. It is used to detect von Willebrand disease, more specifically to highlight an increase affinity in von Willebrand factor (vWF) for GPIb in type 2B and to identify Bernard-Soulier syndrome. Ristocetin reagent can also be used with lyophilized platelets (AG006A) for the Ristocetin co-factor activity assay (vWF:RCo), to assist in the diagnosis of von Willebrand disease.

PRINCIPLE:

When added to platelet-rich plasma (PRP), Ristocetin promotes interaction between von Willebrand factor (vWF) and its platelet receptor, glycoprotein GPIb-V-IX. Ristocetin-induced platelet aggregation (RIPA) is used to measure the patient's PRP aggregation in the presence of various Ristocetin concentrations. Ristocetin can also be used for the vWF:RCo test, using lyophilized platelets.

REAGENTS:

R1 Reagent 1: Ristocetin: lyophilized, contains Tris and stabilizing agents.
3 x 7.5 mg vials.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30 °C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- To ensure optimum test results, we recommend testing the specimens and controls in succession and without interruption.
- For *in vitro* diagnostic use.

H319: Causes serious eye irritation
 H335: May cause respiratory irritation
 H315: Causes skin irritation

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial, gently remove the freeze-drying stopper.

R1 Reagent 1: Ristocetin

For use with the aggregometer:

Reconstitute the contents of each vial with **exactly 0.5 mL distilled water**, shake vigorously until fully dissolved. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally. Homogenize the reagent prior to use.

For use with the analyzer:

Reconstitute the contents of each vial with **exactly 0.625 mL distilled water**, shake vigorously until fully dissolved. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally. Homogenize the reagent prior to use.

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:

- 7 days** at 2-8 °C.
- 8 hours** at room temperature (18-25 °C).
- 2 months** frozen at -20 °C or less*

*Thaw only once, as rapidly as possible at 37 °C, adapting the incubation period to the volume of reagent. The stability of the thawed reagent should be checked under laboratory work conditions.

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8 °C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.
- Saline solution (0.9% NaCl).

Materials:

- SD Medical aggregometer (or equivalent), used as per manufacturer's guidelines, with appropriate stirrers and aggregometry cuvettes.
- Sysmex CS-series analyzer and associated consumables.
- Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI guidelines for further information concerning specimen collection, handling and storage). When screening for thrombocytopeny, patients must not have received any medication known to affect platelet function (e.g.: aspirin) during the previous 10 days. Patients should avoid fatty foods, coffee and dairy products at least 12 hours before collection⁴.

- Specimens:** Human plasma obtained from anti-coagulated blood (trisodium citrate).
- Collection:**

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube. Cover and gently invert 4 to 5 times to mix. **Keep the specimens at room temperature** (~18 to 25 °C). Do not use CTAD tubes for collection.

- Centrifugation:**

Preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP):

- Prepare the PRP by centrifuging the blood specimens onto citrate at 150 x g for 10 minutes at room temperature (18-25 °C).
- Examine the plasma: if any red blood cells are left, re-centrifuge at 150 x g for 5 additional minutes.
- Using a plastic pipette, identify and carefully remove the platelet layer without touching the PRBC (leukocytes and red blood cells) and transfer to an identified tube (PRP). **Seal and store at room temperature.**
- Prepare the PPP by centrifuging the remaining blood specimen at 2000-2500 x g for 15 minutes. Examine the PPP for haemolysis, then transfer to a plastic tube identified 'PPP'. **Seal and store at room temperature.**
- See ISTD guidelines for the PRP platelet count⁵.

PROCEDURE:

Automated method:

The application for Sysmex CS-series analyzers is available on request. **See the application and precautions specific to each analyzer.**

Aggregometer: Ristocetin dilution:

We recommend testing the following concentrations when typing a Willebrand/GPIb anomaly⁵:

Initial concentration (mg/mL)	12	5
Ristocetin 15 mg/mL (µL)	120	60
Saline solution (µL)	30	120
i.e. final concentration in test (mg/mL)	1.2	0.5

Protocol:

The test must be performed within 3 hours of specimen collection.

- Place a stirrer in each cuvette.
- Establish the 100% aggregation point with a cuvette containing **360 µL PPP**.
- Pipette **360 µL** platelet-rich plasma (PRP) into a second cuvette. Incubate for **2 minutes at 37 °C**. Establish the 0% aggregation point with the **PRP**.
- Add **40 µL Ristocetin (5 or 12 mg/mL)** directly into the platelet-rich plasma using a long and fine pipette tip. **Do not inject against the walls of the cuvette.**
- Allow the aggregation profile to develop for 5 to 10 minutes.

Each laboratory can establish and validate its own test protocol and verify the resulting performance under its own specific working conditions (reagents/instruments/test protocol combination).

The user is responsible for validating any changes and their impact on all results.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.

The control should be prepared in the same manner as the specimens. For qualitative platelet aggregation studies, the control may consist of fresh platelet-rich plasma collected from a normal (specified and qualified) donor who has not taken any aspirin or equivalent for the past 10 days and with a history of normal platelet function.

Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new control should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.

Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

The patient's detailed history is required to allow a precise interpretation of the test results. In particular, the patient should be questioned concerning his/her recent treatments, as many prescription or over the counter substance can interfere with platelet aggregation. Substances such as caffeine, tobacco, alcohol, vitamin C, etc. may also affect the results. The results should be analyzed in light of the patient's clinical context.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.

EXPECTED VALUES:

The values expected for each reagent at the various concentrations used, along with the expected performance, must be verified and established by each laboratory under this latter's specific work conditions.

In healthy individuals, aggregation should occur at least at 1.2 mg/mL, but not at 0.5-0.7 mg/mL⁵.

In Bernard-Soulier syndrome and type 3 von Willebrand disease, aggregation is expected to be very weak or non-existent at all concentrations.

In pseudo von Willebrand disease and type 2B von Willebrand disease, the platelets are hyper-sensitive to Ristocetin, leading to aggregation even at low concentrations (i.e. 0.5 mg/mL).

PERFORMANCE:

Example of maximum, normal and abnormal aggregation (%):

	Normal	Abnormal
Maximum aggregation (%)	96	4

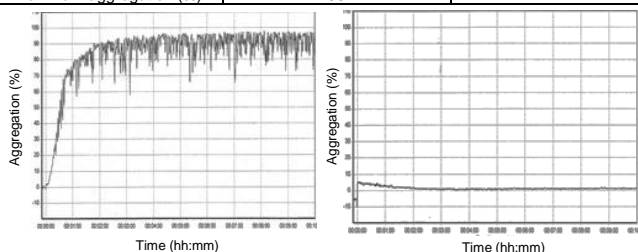


Figure: Example of normal (left) and abnormal (right) aggregation plots with Ristocetin (1.2 mg/mL).

REFERENCES:

1. Yardumian *et al.*, "Laboratory investigation of platelet function: a review of methodology". J Clin Pathol, 39:701-712, 1986.
2. Zhou *et al.*, "Platelet aggregation testing in platelet-rich plasma". AM J Clin Pathol, 123:172-183, 2005.
3. Angiolillo *et al.*, "Basic principles of platelet biology and clinical implications". Circ J, 74:597-607, 2010.
4. McCabe-White and Jennings, "Platelet protocols: research and clinical laboratory procedure". Academic press London, p 35, 1999.
5. Recommendations for the Standardization of Light Transmission Aggregometry: A consensus of the Working Party from the Platelet Physiology Subcommittee of SSC/ISTH. 2013.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to previous version