# RISTOCETIN 25 mg

**For** *in Vitro* **diagnostic use only**

**Kit for evaluation of platelet aggregation on whole blood and on PRP, induced by Ristocetin**

## I. INTENDED USE

Ristocetin is for use in the platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation like von Willebrand Syndrome, the quantitation of von Willebrand factor and the identification of Bernard-Soulier Syndrome.

## II. PRINCIPLE

The ristocetin, a concentration of 1.0-1.5 mg/ml, normal platelet aggregates in rich plasma or citrated whole blood through a mechanism in which the release of endogenous ADP plays only a small role. Ristocetin-induced platelet aggregation at concentrations of 1.2 mg/ml is absent or significantly lower in patients with von Willebrand's syndrome. At concentrations of 1.5 mg/ml there is a lower degree of abnormality. The majority of patients with von Willebrand's syndrome shows a negative response, as well as patients with Bernard-Soulier syndrome.

Aggregation study in whole blood is based on to the evaluation of the electric resistance changes. Two electrodes while immersed in the sample are rapidly covered with platelets which, at the very first contact, appear in shape of monolayer. When the aggregating agent is added, additional platelets tend to attach the monolayer previously formed, determining an impedance increase between the two electrodes.

## III. REAGENTS AND MATERIALS

**Each kit contain:**

1. Ristocetin: Ristocetin A sulphate lyophilised form. Antibiotic isolated from *Nocardia lurida*, containing in excess of 90% Ristocetin A.
2. Diluent A: dilution buffer containing TRIS, pH 7,3.

**MATERIAL REQUIRED BUT NOT SUPPLIED**

- Blood collection tubes, centrifuge tubes, tubes and pipettes for drawing up the blood and the PRP, all in siliconized glass or plastic. Trisodium citrate 3,8%. Cuvettes ad stirrers for aggregometer.
- Aggregometer.

## IV. STORAGE

Store diluent and ristocetin tightly closed in refrigerator (2-8°C),. The kit is stable until expiration date printed on the package label.

## V. SAMPLE COLLECTION

Collect the sample from an antecubital vein without stasis using siliconized needle 18 or 20 G. Immediately transfer blood into a plastic tube containing the anticoagulant (trisodium citrate 3.8%) in the ratio of 1/9 (v/v). The blood can be used as it is for platelet aggregation within 4 hours.

To obtain PRP centrifuge the blood at 160 g for 10 minutes, carefully draw off the supernatant (PRP); centrifuging time and speed depend on the kind of sample; inspect visually the supernatant. In case of red cells presence, centrifuge again. Collect the supernatant (PRP) with the plastic pipette and store in a plastic test tube, identified with the proper label, and carry out a platelet count. Re-centrifuge the remaining citrated blood at 2000 g for 30 minutes and decant the supernatant (PPP). Collect the supernatant (PPP) with the plastic pipette and store in a plastic test tube, identified with the proper label, until the analysis. Dilute PRP with PPP to obtain a plasma with about 300.000 platelet/mm$^3$. Maintain the PRP at room temperature and carry out the test within 4 hours.

## VI. PRP TEST PROCEDURE

Before use, reconstitute a vial of Ristocetin with 0,5 ml of Diluent A. Concentration of work solution: 50 mg/ml. This solution is stable one month at -20°C. To avoid repeated thawing and freezing it is advised to subdivide the solution into aliquots of 0,1 ml and freeze. With the work scheme proposed here, the reagent is sufficient to carryout **33 aggregation test/vial**.

1. Prepare PRP and PPP as described in section V.
2. Add 500 µl (250 µl) of PRP to an aggregation cuvette containing stirring bar and incubate at 37°C for 3 minutes.
3. Add 500 µl (250 µl) of PPP to an aggregation cuvette without stirrer.
4. Place PRP and PPP cuvettes in corresponding instrument sample wells and follow manufacturer's instruction for setting base lines.
5. Add 15 µl (7,5 µl) reconstituted ristocetin to PRP cuvette to obtain a final concentration of 1,5 mg/ml.
6. Record platelet aggregation response for a minimum of 5 minutes.

The figures in parentheses are half volumes that a lot of aggregometers can now handle; using the proper rubber adhesive spacers.

If an aggregation is not obtained or is markedly reduced, (von Willebrand's disease and Bernard-Soulier's syndrome) repeat the test, in order to have a confirmation of the diagnosis, operating as follows:

to 0,4 ml of PRP add 0,1 ml of normal pooled plasma;

add 15 µl of Ristocetin to obtain a final concentration of 1,5 mg/ml;

If an increase in aggregation is recorded, the diagnosis of von Willebrand's disease is confirmed.

## VII. WHOLE BLOOD PROCEDURE

Ristocetin induces optimal platelet aggregation on whole blood at the concentration of 1 mg/ml.

1. Add 500 µl of saline solution and 500 µl of whole blood with anticoagulant in a 1 ml plastic cuvette containing stirring bar and incubate at 37°C for 5 minutes.
2. After connecting the electrode to the socket, put incubated at 37°C for 5 minutes.
3. Alfter incubation, place it in the vial containing the diluted blood. (Place the filaments to the back of aggregometer).
4. Place the cuvette into the reaction well and incubate 2 minutes, holding the door closed. Open the door and pipette 20 µl of ristocetin reconstituted (Warning: avoid formation of air bubbles).
5. Record platelet aggregation.

## VIII. INTERPRETING THE RESULTS

As the normal absolutes values are not available yet, for whole blood aggregation, it is recommended for each laboratory to establish their own normal ranges in order to compare with aggregation curves taken from pathological subjects.

**NOTE:The following Normal Ranges were obtained from various laboratories and pubblications**. They should be used as a guideline only.

| | | | |
|---|---|---|---|
| **Normal values** | Ristocetin in **PRP** - | Concentration 1,5 mg/ml: | % max aggregation 82 - 96%. |
| | Ristocetin in **whole blood** - | Concentration 1,0 mg/ml: | aggregation (ohm) > 5 Ω. <70 sec Lag time |

## IX. LIMITATION OF THE TEST

Carry out the test in subjects on an empty stomach, 8 hours no smoking , not assuming any medical remedies containing Acetylsalicylic Acid for one week or other drugs interfering the platelet aggregation.

## X. PERFORMANCES

This product will perform as described prior to its expiration date when procedural and storage directions are followed.

**Linearity, accuracy, precision.**

Platelet aggregation induced by common agonist (like Ristocetine) is a nonlinear test system for some 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.

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

The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges.

## XI. NOTE

To test at the same time optical test on PRP and the release of ATP with **bioluminescent** technique should work on a **lumi-aggregometer**. (Example 700-2). Refer to the Technical Manual and the instructions in User Manual of instrument.

| **CONTENT** | **REF. 311502D** |
|---|---|
| Ristocetin | 2 x 0,5 ml |
| Diluent A | 1 x 5,0 ml |
| Instruction for use | 1 item |

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| IVD | In Vitro Diagnostic Medical Device | | Temperature limitation | LOT | Batch code (LXXX) | | Manufacturer |
| | Consult Instructions for use | | Use by (year/month) | REF | Catalogue number | | Do not reuse |
| NON STERILE | Non sterile | | Fragile, handle with care | | Keep away from heat | | Keep dry |

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