For in vitro research use only

multiplate®

RISTOtest

for platelet aggregation studies

RISTO reagent kit

2°C 8°C

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0140, MP0240, MP0194

V.3.0-US-RUO Revised 2011-03

This box insert is valid for kit formats MP0140, MP0240 and MP0194 of RISTOtest.

Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer[1]. Ristocetin leads to a vWF (von Willebrand factor)- and GpIb-dependent platelet aggregation.

For the evaluation of qualitative platelet dysfunction and disorders of vWF.

Principle

RISTOtest reagent contains ristocetin. Ristocetin forms a complex with vWF from the sample. This complex binds to the platelet GpIb receptor and triggers platelet activation and aggregation. RISTOtest can be applied in two concentrations: In RISTOhigh test a high concentration of ristocetin (0.77 mg/ml) is applied, which normally induces a strong platelet aggregation. Abolished aggregation in RISTOhigh test can be based on a deficiency of GpIb receptors or vWF. In RISTOlow test a lower concentration of ristocetin (0.2 mg/ml) is applied which normally does not induce a strong aggregation response. A higher than expected aggregation in RISTOlow test may indicate an enhanced aggregation tendency of vWF (vWD type IIb).

Reagents

The reagent is provided in three kit formats:

REF MP0140 – RISTOtest: Ristocetin; 1 x 1.0 ml, lyophilised (10 mg/ml), with 5 micro test tubes for aliquotation.

REF MP0194 – RISTOtest: Ristocetin; 1 x 1.0 ml, lyophilised (10 mg/ml), without micro test tubes for aliquotation.

REF MP0240 – RISTOtest: Ristocetin; 3 x 1.0 ml, lyophilised (10 mg/ml), without micro test tubes for aliquotation.

Reagent preparation

Reconstitute with 1.0 ml of high purity (distilled or deionized) water. Allow to stand at room temperature for 10 minutes and swirl gently to mix – do not shake!

Keep all vials tightly closed when not in use. Minimize exposure to light, air and elevated temperatures.

To achieve maximum stability after reconstitution, pipette at least 100 µl aliquots of the reagent into micro test tubes (MP0094) for daily use.

Storage and stability

Unopened vials of the RISTOtest reagent must be stored at 2-8°C. The reagent is stable until the expiry date printed on the vial label when stored under these conditions. If reconstituted reagent is not aliquoted into micro test tubes, the original vial should be stored in an upright position.

Stable 7 days after reconstitution when stored at 2-8°C. When stored at < -20°C stable for 4 weeks. Stable for 24 hours at room temperature after one time thawing.

Warnings and precautions

General precautions should be followed when handling specimen and all materials, e.g. wear gloves, minimize exposure of specimen and reagents to the skin. Dispose of all waste materials according to the local regulations.

Sample collection

Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Also avoid foam formation in the blood collection tube. Gently invert the collection tube to ensure complete mixing of the content. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

The anticoagulant used for blood sample collection significantly affects the results of the test[2]. The use of hirudin as the sample anticoagulant is recommended with a final concentration of 25 µg/ml. Recombinant hirudin is diluted to a concentration of 2.5 mg/ml and applied into the blood collection tube in a ratio of 1:100 (e.g. 30 µl hirudin solution for 3 ml of blood).

Alternatively commercial hirudin tubes (MP0600), standard lithium-heparin tubes or citrated tubes (3.2% citrate) may be used. Always ensure citrate blood collection tubes are filled to the indicated fill volume in order to avoid excessive citrate levels.

The blood collection system must be standardised at each center. It is only possible to compare the results of an individual sample with reference ranges when the same sample anticoagulant (i.e. heparin, citrate or hirudin) is employed.

Performance of the analysis

Samples should be analyzed within the period of 0.5-3 hours after blood collection. Follow the instructions in the Multiplate® user manual and short instructions manual.

Test procedure for RISTOhigh:

300 µl saline 0.9%, preheated at 37°C
+ 300 µl whole blood (hirudin blood / heparin blood / citrated blood, room temperature)
→ 3 minutes incubation
+ **50 µl** RISTOtest reagent
→ Start test → 6 minutes measuring time

Test procedure for RISTOlow:

300 µl saline 0.9%, preheated at 37°C
+ 300 µl whole blood (hirudin blood / heparin blood / citrated blood, room temperature)
→ 3 minutes incubation
+ **12 µl** RISTOtest reagent
→ Start test → 6 minutes measuring time

Final concentrations: 0.77 mg/ml ristocetin (RISTOhigh) or 0.2 mg/ml ristocetin (RISTOlow)

It is important to pay close attention to temperatures and incubation times. The use of non-preheated saline solution or the introduction of shorter incubation times may skew results.

The saline (NaCl 0.9%) must not contain any additives such as methyl ester. This can cause false-positive results.

When using the Multiplate® electronic pipette follow the software instructions displayed by the Multiplate®.

Note: A partial recalcification of the sample when citrated blood is analyzed (as recommended for the TRAPtest or ADPtest) may lead to disaggregation during the analysis. The reason for this phenomenon is unclear. The use of saline-$CaCl_2$ diluent solution (instead of the use of saline solution) for the analysis of RISTOtest is therefore not recommended.

Literature

[1] Sibbing D, Braun S, Jawansky S, Vogt W, Mehilli J, Schömig A, Kastrati A, von Beckerath N. Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. Thromb Haemost 2008; 99(1): 121-6.

[2] Tóth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: A new device to measure platelet aggregation in whole blood. Thromb Haemost 2006; 96(6): 781-8.

Manufacturer

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