**For in vitro research use only**

**RISTOtest**

for platelet aggregation studies

**RISTO reagent kit**

Valid for REF MP0140, MP0240, MP0194

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**Manufacturer**

Verum Diagnostica GmbH
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**Distributor**

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**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 exposure of specimen and reagents to the skin. Dispose of all waste materials according to the local regulations.

**Intended use**

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer¹. Ristocetin leads to a vWF (von Willebrand factor)- and Gp Ib-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 Gp Ib 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 Gp Ib 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 llb).

**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.

**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!

**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 test². 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.

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**Reference**
