## For in vitro research use only

multiplate®

# vWF reagent

for platelet aggregation studies

**vWF reagent kit**

2°C 8°C

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0548

V.1.0-US-RUO Revised 2012-02

This box insert is valid for kit format MP0548 of vWF reagent.

### Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer[1] in RISTOconfirm assay.

In the RISTOconfirm assay RISTOtest is performed with the addition of vWF reagent in order to determine whether additional vWF improves or corrects RISTOtest in the individual patient sample.

### Background

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 RISTOtest high a high concentration of ristocetin (0.77 mg/ml) is applied, which normally induces a strong platelet aggregation. Attenuated aggregation in RISTOtest high can be based on a deficiency of GpIb receptors or vWF.

In RISTOtest low 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 RISTOtest low may indicate an enhanced aggregation tendency of vWF (vWS type IIb).

Different disorders can lead to low aggregation in RISTOtest:

- GpIb deficiency (Bernard-Soulier syndrome)
- deficiency in vWF (vWD)
- platelet inhibition e.g. by aspirin
- thrombocytopenia

By the addition of vWF reagent a deficiency of vWF is corrected, whereas the other disorders remained unchanged.

This may facilitate the differentiation of disorders leading to low aggregation in RISTOtest.

| | RISTOtest | RISTOconfirm |
|---|---|---|
| **Normal** | normal | normal |
| **GpIb deficiency (Bernard-Soulier syndrome)** | abnormal | abnormal * |
| **Aspirin** | abnormal | abnormal |
| **Thrombocytopenia** | abnormal | abnormal |
| **vWD** | abnormal | normal |

** theoretical, not proven yet*

### Reagents

The reagent is provided in the following format:

REF MP0548 – vWF reagent: vWF reagent; 1 x 1.0 ml, lyophilised (7.5 U/ml) with 5 micro test tubes for aliquotation.

### Reagent preparation

Reconstitute the vWF reagent with 1.0 ml of high purity (distilled or deionized) water (vWF concentration: 7.5 U/ml). Allow to stand at room temperature for 10 minutes and swirl gently to mix – do not shake! The solution should be clear and colourless.

**Note:** Due to risk minimization procedures the vacuum in the vials was replaced by an inert gas.

Keep all vials tightly closed when not in use. It is advisable to minimize exposure to light, air and elevated temperatures.

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

### Storage and stability

Unopened vials of vWF 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. Store the reconstituted reagent at 2-8°C. If reconstituted reagent is not transferred into micro test tubes, the original vial should be stored in an upright position.

### 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

The anticoagulant used for the blood collection significantly affects results of the tests[2]. The use of hirudin as sample anticoagulant is recommended with a final concentration of 25 µg/ml. For this 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 that citrated blood collection tubes are filled to the indicated fill volumes, in order to avoid enhanced citrate levels.

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

Avoid foam formation in the sample tube during blood collection. Gently invert the sample tube to ensure complete mixing of the content. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

### 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, short instructions manual and the box insert of RISTOtest.

Test procedure for RISTOtest with addition of vWF reagent (RISTOconfirm)

| |
|---|
| 280 µl saline 0.9%, preheated at 37°C |
| + **20 µl** vWF reagent (7.5 U/ml) |
| + 300 µl whole blood (hirudin- / heparin-anticoagulated blood or citrated blood, room temperature) |
| → 3 minutes incubation |
| + 50 µl RISTOtest reagent |
| → Start test → 6 minutes measuring time |

Final concentration of vWF: 0.23 U/ml

Final concentration of ristocetin: 0.77 mg/ml

Follow exactly this procedure. The use of non-preheated saline diluent solution or shorter incubation times may skew results.

Furthermore the saline must not contain any additives such as methyl ester.

### Quality control

Results obtained in RISTOconfirm should be compared with these obtained in RISTOtest high (without the addition of vWF).

### 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.


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