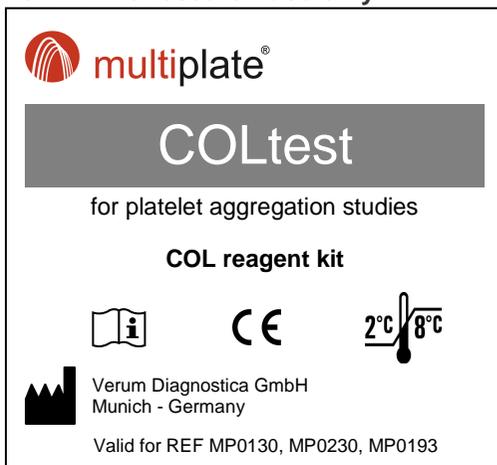


For in vitro research use only



This box insert is valid for kit formats MP0130, MP0230 and MP0193 of COLtest.

Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer¹. Sensitive to a cyclooxygenase inhibition, GpIIb/IIIa antagonists and deficiency of GpIIb/IIIa receptors.

For the evaluation of qualitative platelet disorders and platelet function inhibition.

Principle

The COLtest reagent contains collagen (type I), which activates the platelets via their collagen receptors. Following binding of collagen to its receptors, arachidonic acid is released, which is the substrate of the platelet enzyme cyclooxygenase. Cyclooxygenase transforms arachidonic acid into thromboxane A₂, a potent platelet activator. With a blockade of cyclooxygenase the formation of thromboxane A₂ is inhibited and therefore inhibited platelet activation is usually detected.

Reagents

The reagent is provided in three kit formats:

[REF] MP0130 – COLtest: Collagen; 1 x 1.0 ml, lyophilised (activity equivalent to 100 µg/ml), with 5 micro test tubes for aliquotation.

[REF] MP0193 – COLtest: Collagen; 1 x 1.0 ml, lyophilised (activity equivalent to 100 µg/ml), without micro test tubes for aliquotation.

[REF] MP0230 – COLtest: Collagen; 3 x 1.0 ml, lyophilised (activity equivalent to 100 µg/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 (MP0093) for daily use.

Storage and stability

Unopened vials of COLtest 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. Do not freeze the reconstituted reagent.

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.

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 hirudin or heparin blood:

300 µl saline 0.9%, preheated at 37°C
+ 300 µl whole blood (room temperature)
→ 3 minutes incubation
+ 20 µl COLtest reagent
→ Start test → 6 minutes measuring time

Test procedure for citrated blood:

300 µl saline-CaCl ₂ (MP0530), preheated at 37°C
+ 300 µl whole blood (room temperature)
→ 3 minutes incubation
+ 20 µl COLtest reagent
→ Start test → 6 minutes measuring time

The final concentration of collagen equates to an activity of 3.2 µg/ml.

It is important to pay close attention to temperatures and incubation times. The use of non-preheated saline or saline-CaCl₂ diluent solution (MP0530) or the introduction of shorter incubation times may skew results.

The saline (NaCl 0.9%) must not contain any additives such as methyl ester.

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

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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