For in vitro research use only

multiplate®

PAR-4 test

for platelet aggregation studies

PAR-4 test reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0549

V.2.0-US-RUO Revised 2012-02

This box insert is valid for kit format MP0549 of PAR-4 test (PAR-4 Agonist; H-AYPGKF-OH).

Principle

Thrombin is one of the most potent platelet agonists. It interacts with 2 protease-activated receptors (PARs) on the surface of human platelets – PAR-1 and PAR-4. The expression profiles of PARs on platelets differ between humans and nonprimates. PAR-1 is believed to be the primary receptor for thrombin in humans. In contrast, mouse platelets for example lack PAR-1 and largely signal through PAR-4 in response to thrombin.

Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer[1] to evaluate the qualitative platelet function in species expressing PAR-4 receptors. In particular PAR-4 test can be used for determination of PAR-mediated aggregation in species that do not express PAR-1 (stimulated by TRAP-6).

Reagents

The reagent is provided in the following format:

REF MP0549 – PAR-4 test: PAR-4 Agonist;
1 x 1.0 ml, lyophilised (20 mM) with 5 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! 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 the provided micro test tubes for daily use. Store the reconstituted reagent as recommended below.

Storage and stability

Unopened vials of PAR-4 test 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 transferred into micro test tubes, the original vial should be stored in an upright position.

Stable 14 days after reconstitution when stored at 2-8°C. Stable 4 weeks after reconstitution when stored at < -20°C. Stable for 24 hours after 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

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 contents. 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 and short instructions manual.

Test procedure for Multiplate® test cells (MP0020):

300 µl saline 0.9%, preheated at 37°C
+ 300 µl anticoagulated whole blood (room temperature)
→ 3 minutes incubation
+ 20 µl PAR-4 test
→ Start test → 6 minutes measuring time

Final concentration of PAR-4 test: 645 µM

Test procedure for Multiplate® **mini test cells** (MP0021):

175 µl saline 0.9%, preheated at 37°C
+ 175 µl whole blood (room temperature)
→ 3 minutes incubation
+ 12 µl PAR-4 test
→ Start test → 6 minutes measuring time

Final concentration of PAR-4 test: 662 µM

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

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

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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