For in vitro research use only

multiplate®

Prostaglandin E1

for platelet aggregation studies

PGE1 reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0160, MP0260, MP0540

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This box insert is valid for kit formats MP0160, MP0260 and MP0540 of Prostaglandin E1.

For the assessment of ADPtest HS.
For the assessment of positive (i.e. abnormal) controls of the ADPtest.

Prostaglandin E1 (PGE1) is used in combination with the **ADPtest** reagent (MP0120).

Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer[1]. For the evaluation of qualitative platelet defects as well as platelet function inhibition. PGE1 enhances the sensitivity of ADPtest to platelet function inhibition especially to the effect of clopidogrel. Moreover, the addition of a higher dose of PGE1 into the sample can induce an abnormal aggregation in ADPtest, which allows the performance of an abnormal control (positive control).

Principle

PGE1 is a natural platelet inhibitor which triggers an increase in cAMP levels in the platelet. cAMP is a so-called second messenger, i.e. an intracellular signalling molecule. A decrease of the cAMP level in the platelet leads to platelet activation. An increase of the cAMP level counteracts platelet activation.

The addition of 20 µl PGE1 to the ADPtest (9.4 nM PGE1 final conc.) induces a moderate inhibition of platelet activation in healthy normal blood samples, but a significant increase of sensitivity of the ADPtest to the platelet inhibition by clopidogrel.

The addition of 50 µl PGE1 into ADPtest (22 nM PGE1 final conc.) normally induces a strong inhibition of ADP induced aggregation (positive control for ADPtest).

Reagents

The reagent is provided in three kit formats:

REF MP0160 – PGE1: Prostaglandin E1; 1 x 1.0 ml, lyophilised (300 nM), with 5 micro test tubes for aliquotation.

REF MP0540 – PGE1: Prostaglandin E1; 1 x 1.0 ml, lyophilised (300 nM), without micro test tubes for aliquotation.

REF MP0260 – PGE1: Prostaglandin E1; 3 x 1.0 ml, lyophilised (300 nM), 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! 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. 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 (MP0096) for daily use.

Storage and stability

Unopened vials of PGE1 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) or standard lithium-heparin tubes may be used for the analysis. There is no experience for this reagent with the use of citrated blood.

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 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.

Performance of ADPtest HS

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** PGE1
+ 20 µl ADPtest reagent
→ Start test → 6 minutes measuring time

Final concentration: 9.4 nM PGE1

Performance of a positive control of ADPtest (test name: ADPtest abn.control)

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
+ **50 µl** PGE1
+ 20 µl ADPtest reagent
→ Start test → 6 minutes measuring time

Final concentration: 22 nM PGE1

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®.

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

Verum Diagnostica GmbH
Munich - Germany
Phone: +49-89-125556-0
www.multiplate.us
service@verumdiagnostica.com

Distributor

DiaPharma Group, Inc.
8948 Beckett Road, West Chester
OH 45069, USA
Customer Service 800-526-5224
Technical Service 800-447-3846
www.diapharma.com
info@diapharma.com