For in vitro research use only

multiplate®

GpIIb/IIIa Antagonist

for use as quality control in platelet aggregation studies

GpIIb/IIIa Antagonist reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0280, MP0520

V.3.0-US-RUO Revised 2011-03

This box insert is valid for kit formats MP0280 and MP0520 of GpIIb/IIIa Antagonist.

Intended use

For in vitro research use only. Reagent for use as quality control in platelet aggregation studies on the Multiplate® analyzer[1,2]. GpIIb/IIIa antagonist inhibits the fibrinogen receptors of the platelets thus preventing their adhesion onto the sensor wires of the Multiplate® test cell. The reagent is employed in combination with the Multiplate® activating reagents TRAPtest, ASPItest, COLtest and ADPtest. Addition of GpIIb/IIIa antagonist to the blood sample leads to strongly reduced aggregation in TRAPtest, ADPtest, ASPItest and COLtest.

Principle

GpIIb/IIIa antagonist contains a synthetic inhibitor of the platelet GpIIb/IIIa receptor with a molecular weight of 495 g/mol at a concentration of 50 µg/ml.

Blocking the GpIIb/IIIa receptor leads to abolished aggregation in the Multiplate® tests. This allows the assessment of a positive control (strongly inhibited aggregation) in all tests.

Furthermore unspecific effects of chemicals present in the sample onto the Multiplate® sensors can be elucidated (e.g. addition of oxidative substances into the blood).

Explanation: Normally when the GpIIb/IIIa antagonist is added to the sample no significant impedance change during the analysis is recorded, because the binding of platelets onto the sensor wires is blocked. If a significant increase or decrease of impedance is still recorded when the GpIIb/IIIa antagonist is added to the sample, this suggests unspecific direct effects of substances in the blood or substances in reagents (that were not developed by Verum Diagnostica GmbH) onto the sensor wires (e.g. oxidating effects).

Reagents

The reagent is provided in two kit formats:

REF MP0520 – GpIIb/IIIa antagonist: synthetic GpIIb/IIIa antagonist (molecular weight 495 g/mol in a concentration of 50 µg/ml); 1 x 0.5 ml, liquid, ready for use.

REF MP0280 – GpIIb/IIIa antagonist: synthetic GpIIb/IIIa antagonist (molecular weight 495 g/mol in a concentration of 50 µg/ml); 3 x 0.5 ml, liquid, ready for use.

Reagent preparation

The reagent is ready for use. Keep all vials tightly closed when not in use. Minimize exposure to light, air and elevated temperatures.

Storage and stability

Unopened vials of the GpIIb/IIIa antagonist 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. Vials should be stored in an upright position.

Stable 30 days after opening when stored at 2-8°C.

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), 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. Refer to the instructions in the Multiplate® user manual and short instructions manual.

Pipette procedures

Add 20 µl of GpIIb/IIIa antagonist into the sample before the addition of the agonist.

Example:

TRAPtest with the addition of GpIIb/IIIa antagonist

Test procedure for hirudin or heparin blood:

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

Test procedure for citrated blood:

300 µl saline-$CaCl_2$ (MP0530), preheated at 37°C
+ 20 µl GpIIb/IIIa antagonist
+ 300 µl whole blood (room temperature)
→ 3 minutes incubation
+ 20 µl TRAPtest reagent
→ Start test → 6 minutes measuring time

Final concentration: 1.6 µg/ml GpIIb/IIIa antagonist

It is important to pay close attention to temperatures and incubation times. The use of non-preheated saline or saline-$CaCl_2$ 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.

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