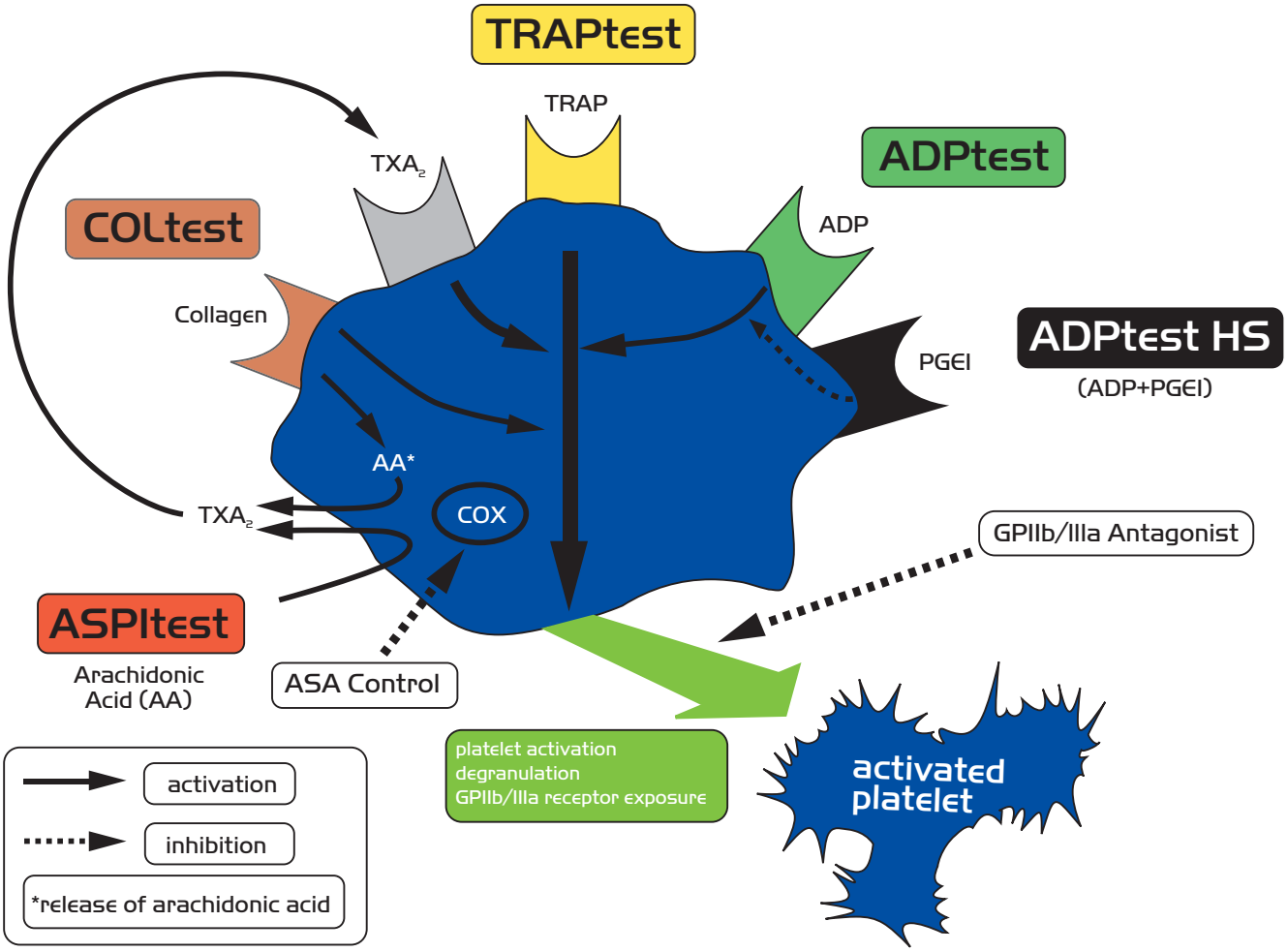

PACKAGE INSERTS

THE MULTIPLATE® PLATELET FUNCTION ANALYZER

for research use only

diaPharma 8948 Beckett Road | West Chester, Ohio 45069

www.diapharma.com | info@diapharma.com | 1.800.526.5224

For in vitro research use only

multiplate®

Hirudin Blood Tube
(Double Wall) Premium
Vacuette system

Blood collection system
Anticoagulant: recombinant hirudin

REF **MP0600** 50 x 3.0 ml
REF **MP0601** 10 x 3.0 ml

4°C 25°C

Verum Diagnostica GmbH
Munich - Germany

V.2.0-US-RUO Revised 2012-11

Intended use

For in vitro research use only. For use in platelet aggregation studies with the Multiplate® platelet function analyzer to evaluate qualitative platelet disorders at physiological calcium conditions.

Hirudin, a thrombin inhibitor allows anticoagulation of blood without interference with physiological calcium levels.

The specified concentration of hirudin in the blood collection tubes is > 15 µg/ml.

Packages

The blood tubes are available in the following quantities:

REF MP0600 – 50 x 3.0 ml tubes
anticoagulant: recombinant hirudin

REF MP0601 – 10 x 3.0 ml tubes
anticoagulant: recombinant hirudin

Storage and stability

Store tubes at 4-25°C. Avoid exposure to direct sunlight.

Note: Deviation from recommended storage conditions may lead to impairment of the tube quality.

Warnings and precautions

Do not use tubes if foreign matter is present!

1. Handle all biological samples and blood collection "sharps" (lancets, needles, luer adapters, and blood collection sets) according to the policies and procedures of your facility.
2. Obtain appropriate medical attention in the case of any exposure to biological samples (for example through a puncture injury), since they may transmit HIV, viral hepatitis, or other blood-borne pathogens.
3. Discard all blood collection "sharps" in biohazard containers approved for their disposal.
4. Transferring a sample from a syringe to a tube is not recommended.
5. If blood is collected through an intravenous (IV) line, ensure that the line has been cleared of IV solution before beginning to fill blood collection tubes. This is critical to avoid erroneous laboratory data from IV fluid contamination.
6. Do not use tubes after their expiration date.

Venipuncture Technique and Specimen Collection

Equipment required for specimen collection

1. The appropriate amount of hirudin tubes.
2. Labels for positive patient identification of samples.
3. Butterfly blood collection system, needle and tube holder.
4. Practice general safety precautions, using gloves and appropriate apparel for protection from exposure to blood-borne pathogens.
5. Alcohol swab for cleansing site.
6. Tourniquet.
7. Adhesive plaster or bandage.
8. Sharps disposal container for safe disposal of used needle.

Prevention of backflow

To prevent backflow from the tube into the patient's arm, observe the following precautions:

1. Place patient's arm in a downward position.
2. Hold tube with the cap uppermost.
3. Release tourniquet as soon as blood starts to flow into tube.
4. Make sure tube contents do not touch cap or end of the needle during venipuncture.
5. Use of a butterfly system or similar catheter between the patient and the blood collection tube.

Specimen collection procedure

1. Remove the cover over the valve section of the needle.
2. Thread the needle firmly into the tube holder. Attach the butterfly blood collection system with its luer adapter part.
3. Apply tourniquet
4. Prepare venipuncture site with an appropriate antiseptic.
5. Place patient's arm in a downward position.
6. Remove needle shield of the butterfly system.
7. Perform venipuncture.

Note: It is recommended to use a no-additive discard tube as first tube.

8. Push tube into the holder and onto the needle valve puncturing the rubber diaphragm. Centre tubes in holder when penetrating the cap to prevent sidewall penetration and subsequent premature vacuum loss.
9. When the tube is full and blood flow ceases, remove it from holder and **gently invert the tubes at least 5 times** to reach a proper mix of anticoagulant and blood.

Note: Inadequate mixing of tubes may result in platelet clumping, clotting and/or incorrect test results.

10. Optionally, fill further tubes by repeating steps 8 and 9. Follow your facility's protocol and the above named warnings and precautions in order to terminate specimen collection procedure accordingly.

The tube cap can be removed by a simple and cautious pull action.

Literature

In literature the use of citrate as anticoagulant for platelet function analysis is discussed controversially. Concerns are, that citrate depletes calcium and by reducing free calcium levels inhibits platelet function.

In several publications hirudin was used as anticoagulant in concentration ranges of 5-75 µg/ml.

References

Kalb ML, Potura L, Scharbert G, Kozek-Langenecker SA. The effect of ex vivo anticoagulants on whole blood platelet aggregation. Platelets 2009; 20(1):7-11.

Johnson A, Dovlatova N, Heptinstall S. Multiple electrode aggregometry and P2Y(12) antagonists. Thromb Haemost 2008; 99(6):1127-1129.

Storey RF, Wilcox RG, Heptinstall S. Differential Effects of Glycoprotein IIb/IIIa Antagonists on Platelet Microaggregate and Macroaggregate Formation and Effect of Anticoagulant on Antagonist Potency. Circulation 1998; 98:1616-1621.

Wallen NH, Ladjevardi M, Albert J, Broijersen A. Influence of different anticoagulants on platelet aggregation in whole blood; a comparison between citrate, low molecular mass heparin and hirudin. Thromb Res 1997; 87(1):151-157.

Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, Gaussem P. Adenosine Diphosphate–Induced Platelet Aggregation Is Associated With P2Y12 Gene Sequence Variations in Healthy Subjects. Circulation 2003; 108(8):989-995.

Belcher PR, Muriithi EW, Day SP, Wheatley DJ. Platelet aggregatory responses to low-dose collagen are maintained in hirudin-anticoagulated whole blood for 24 h when stored at room temperature. Platelets 2001; 12(1):34-36.

Manufacturer

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For in vitro research use only

multiplate®

Liquid Control Set

quality control verification
of impedance aggregometry

REF **MP0720**

2 x 4.0 ml Solution 1
1 x 2.0 ml Solution 2

CE 2°C 8°C

Verum Diagnostica GmbH
Munich - Germany

V.4.0-US RUO Revised 2011-05

Intended use

For use as an assayed quality control verification of the resistance measure of impedance aggregometry.

Principle

The signal reaction of the Multiplate® analyzer is based on the detection of the change of electrical resistance during the measurement. Using artificial control material allows the quality assessment of the detection mechanism.

The liquid control set consists of two fluids, "Solution 1" and "Solution 2", of different ionic strengths.

Mixing of the fluids in various proportions results in a change of electrical conductivity, which is recorded as a change in impedance in the Multiplate® analyzer.

The set contains enough control material to test the level 1 and level 2 control in all channels of the Multiplate® analyzer.

Materials Provided

MP0720: Liquid Control Set

Solution 1: 2 x 4.0 ml
Solution 2: 1 x 2.0 ml

Materials required but not provided

1. Aggregometer test cells with stir bars
2. Pipettes – 100 µl to 1 ml required

Instrumentation

The Liquid Control will perform as described when used with the Multiplate® aggregometer. Follow the manufacturer's instructions.

Reagent Preparation

The reagents are provided in ready-to-use form.

Storage and stability

Liquid quality control solutions must be stored at 2-8°C. The set is stable until the expiry date printed on the tube label when stored under these conditions.

Note: Opened tubes must be used within 24 hours of opening.

Warnings and precautions

For in-vitro research use only. General precautions should be followed when handling all materials, e.g. wear gloves, minimize exposure of reagents to the skin. Dispose of all waste materials according to the local regulations.

Test procedure

Preheat the reagents for 20 min at 37°C in the preheating positions of the Multiplate® analyzer prior to use. Run measurements for level 1 and level 2 controls as follows:

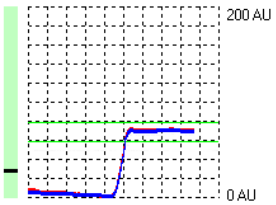
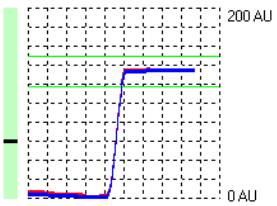
Test procedure for liquid quality controls	
Level 1	**Level 2**
Load all 5 channels with Multiplate® test cells	
Attach the sensor cables to the test cells	
Add 600 µl of "Solution 1" into each channel	
3 min incubation phase (select <F2:Start timer>)	
Select **<F3: Start test>** for all channels	
select <F2:Start timer> again, and **wait for the first 3 min of measuring time**	
Add 100 µl of "Solution 2" **onto** the surface of "Solution 1".	**Add 200 µl** of "Solution 2" **onto** the surface of "Solution 1".
Do not immerse the pipette tip into "Solution 1" to avoid air bubbles.	
Wait for the completion of 6 min test time	
Print out and compare aggregation results with expected values	

Note: It is important to precisely follow this procedure. The use of non-preheated solutions or shorter incubation times may skew results. It is important that "Solution 2" is pipetted **onto** the surface of "Solution 1".

When using the Multiplate® electronic pipette in auto mode follow the test instructions displayed by the Multiplate® software.

Expected values

Expected values for the liquid quality control analyses, which are marked by two horizontal green lines in the graphic window, are as follows:

Expected values of liquid quality control
Level 1
59.1-78.7 AU

Level 2
117.9-149.6 AU


If results of a liquid control analysis are not within the expected range, repeat the analysis. If a channel's results repeatedly fall outside of the expected range lock the appropriate channel in the Multiplate® software (menu *Multiplate -> Channel administration*) and contact the manufacturer or local Multiplate® representative for service.

Limitations

The liquid quality control is an artificial quality control of the Multiplate® analysis. The quality of the electronic parts and sensors of the Multiplate® system as well as the quality of the optional electronic pipette is assessed. The liquid control does not assess the appropriate stirring of the sample or the proper performance of aggregation reagents.

Literature

[1] Köppen K., Wittwer M., Calatzis A., Spannagl M.: External quality control of impedance aggregometry analysis: a feasibility study; poster abstract P-14-13; GTH congress, Society of Thrombosis and Haemostasis annual meeting, Wiesbaden 2008

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For in vitro research use only

multiplate®

NaCl/$CaCl_2$

for platelet aggregation studies

REF **MP0530** 10 x 5.0 ml



non-sterile

Verum Diagnostica GmbH
Munich - Germany

V.2.0-US-RUO Revised 2011-04

Product description

The NaCl/$CaCl_2$ diluent is a mix of calcium chloride (3 mM) and physiological saline (0.9%).

Intended use

For use as a sample diluent in platelet aggregation studies with the Multiplate® platelet function analyzer under reduced calcium concentrations associated with the use of citrated blood samples.

Principle

Citrate depletes the calcium in a blood sample. Reduced calcium levels may lead to falsely reduced aggregation levels in platelet function testing[1]. Therefore it is recommended to partially re-calcify citrated samples with this diluent solution for testing in the Multiplate®. The 3 mM $CaCl_2$ diluent solution enhances the calcium levels of the sample while maintaining the anticoagulant effect of the citrate.

Materials provided

MP0530: NaCl/$CaCl_2$ diluent, 10 x 5.0 ml tubes

Instrumentation

The NaCl/$CaCl_2$ diluent will perform as described when used with the Multiplate® aggregometer. Follow the manufacturer's instructions.

Reagent preparation

The NaCl/$CaCl_2$ diluent is packaged ready-for-use. Prior to use in a test, the diluent must be pre-warmed to 37°C by placing the tube in the pre-heating position of the Multiplate® instrument for a minimum of 10 minutes.

Storage and stability

Store tubes at 2-8°C. The product is stable until the expiry date on the tube label.

Warnings and precautions

Ensure proper storage conditions. Use the contents of opened tubes within one week of opening. Discard the tube if suspect of contamination with other substances.

Sample collection

Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Collect samples into sterile evacuated tubes with non-wettable lining containing 1/10 volume of 3.2% buffered sodium citrate. Avoid foam formation in the blood collection tube. Always ensure citrated blood collection tubes are filled to the indicated fill volume, in order to avoid excessive citrate levels. Gently invert the collection tube 4 to 5 times to ensure complete mixing of the contents. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

Test procedure

Refer to the test procedures as described in the box insert of the Multiplate® test reagent or Multiplate® user's manual. The final concentration of $CaCl_2$ in the Multiplate® sample is 1.5 mM.

Literature

[1] Calatzis A, Wittwer M, Krueger B: A new approach to platelet function analysis in whole blood – The Multiplate Analyzer. Platelets 2004, 15(8), 485-486

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The NaCl/$CaCl_2$ diluent is a mix of calcium chloride (3 mM) and physiological saline (0.9%).
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MP0530: NaCl/$CaCl_2$ diluent, 10 x 5.0 ml tubes
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Reagent preparation
The NaCl/$CaCl_2$ diluent is packaged ready-for-use. Prior to use in a test, the diluent must be pre-warmed to 37°C by placing the tube in the pre-heating position of the Multiplate® instrument for a minimum of 10 minutes.
Storage and stability
Store tubes at 2-8°C. The product is stable until the expiry date on the tube label.
Warnings and precautions
Ensure proper storage conditions. Use the contents of opened tubes within one week of opening. Discard the tube if suspect of contamination with other substances.
Sample collection
Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Collect samples into sterile evacuated tubes with non-wettable lining containing 1/10 volume of 3.2% buffered sodium citrate. Avoid foam formation in the blood collection tube. Always ensure citrated blood collection tubes are filled to the indicated fill volume, in order to avoid excessive citrate levels. Gently invert the collection tube 4 to 5 times to ensure complete mixing of the contents. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.
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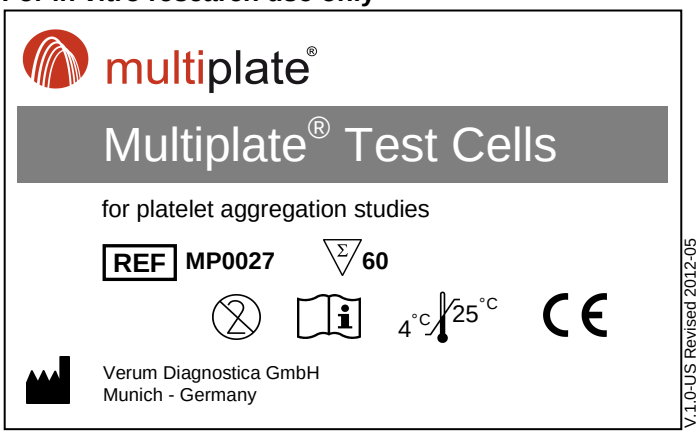

Intended use

For single use in platelet aggregation studies with the Multiplate® platelet function analyzer with sample volumes of 300 µl whole blood.

Storage and stability

Store the product at 4-25°C. Avoid exposure to air, moisture or direct sunlight. Reseal opened boxes of primary PET packaging accordingly. **Use test cells of opened PET boxes within one month after opening.**

Warnings and precautions

Test cells are single use products. Do not reuse. Do not use volumes for analyses below the minimum volume of 600 µl.

General precautions should be followed when handling specimen and all contaminated materials, e.g. wear gloves, minimize exposure of specimen and reagents to the skin. Dispose of all waste materials according to the local regulations.

Note: Avoid touching electrode wires when handling new unused test cells and make sure that the stirring bar freely rotates at the bottom of the test cell when inserted in the measuring position.

Performance of the analysis

Follow the instructions in the Multiplate® user manual, short instructions manual and instructions for use (IFU) for the Multiplate® reagents.

Test procedure

The minimum volume of the test cell is 600 µl.

Add 300 µl saline 0,9% (preheated at 37°C)
Add 300 µl whole blood (preferably hirudin or lithium heparin anti-coagulated blood, stored at room temperature)
→ 3 minutes incubation time
Add the appropriate amount of agonist
→ Start test → 6 minutes measuring time

Manufacturer

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For in vitro research use only

multiplate®
Multiplate® Test Cells
for platelet aggregation studies
REF MP0027
Σ 60
4°C 25°C
Verum Diagnostica GmbH
Munich - Germany
V.1.0-US Revised 2012-05

Intended use

For single use in platelet aggregation studies with the Multiplate® platelet function analyzer with sample volumes of 300 µl whole blood.

Storage and stability

Store the product at 4-25°C. Avoid exposure to air, moisture or direct sunlight. Reseal opened boxes of primary PET packaging accordingly. **Use test cells of opened PET boxes within one month after opening.**

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Test cells are single use products. Do not reuse. Do not use volumes for analyses below the minimum volume of 600 µl.

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For in vitro research use only

multiplate®

ADPtest

for platelet aggregation studies

ADP reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0120, MP0220, MP0192

V.4.0-US-RUO Revised 2012-02

Product description

The ADPtest reagent is a lyophilized preparation of adenosine-5'-diphosphate, stock concentration 0.2 mM.

Intended use

The ADPtest reagent is for use in routine platelet aggregation studies for the evaluation of normal platelet function.

Principle

When added to a platelet sample, ADP triggers platelet activation via a platelet's ADP receptors. Exposure to exogenous ADP will cause normal platelets to release endogenous ADP from their granules and result in irreversible aggregation.

Materials provided

The reagent is provided in one of three kit formats:

MP0120: ADPtest reagent, 1 x 1.0 ml,
with 5 color coded micro test tubes for aliquotation.

MP0192: ADPtest reagent, 1 x 1.0 ml.

MP0220: ADPtest reagent, 3 x 1.0 ml.

Materials required but not provided

1. Platelet aggregometer
2. Purified water (distilled or deionized)
3. Aggregometer test cells with stir bars
4. Micropipettes – 0.5 µl to 100 µl required for reagents
5. Pipettes – 100 µl to 1 ml required for blood samples
6. Physiological saline (NaCl 0.9%) for irrigation/$CaCl_2$ (MP0530) for the dilution of whole blood sample

Instrumentation

ADP will perform as described when used on the Multiplate® Aggregometer. Follow the manufacturer's instructions.

Reagent preparation

Reconstitute each vial of ADP 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 colorless.

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

Storage and stability

Lyophilized Reagent: until the expiry date printed on the vial label when stored at 2-8°C.

Reconstituted Reagent: for 7 days when stored at 2-8°C. When stored at < -20°C the reconstituted reagent is stable for 4 weeks. Reconstituted vials should remain tightly closed when not in use. It is advisable to minimize exposure to light, air and elevated temperatures.

For optimal handling, reconstituted reagent may be aliquoted and the aliquots stored frozen at < -20°C.

Freshly thawed aliquots are stable for 24 hours at room temperature after one freeze thaw cycle.

Warnings and precautions

The ADPtest reagent is for In-vitro-research use only and not for injection or ingestion. Observe standard precautions when handling test specimens and all test materials. Dispose of all waste materials according to local regulations.

Sample collection

Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Collect samples into sterile evacuated tubes with non-wettable lining containing 1/10 volume of 3.2% buffered sodium citrate. Avoid foam formation in the blood collection tube. Always ensure citrated blood collection tubes are filled to the indicated fill volume, in order to avoid excessive citrate levels. Gently invert the collection tube 4 to 5 times to ensure complete mixing of the contents. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

Test procedure

Final working concentration: 6.5 µM ADP

1. Insert a disposable test cell with stir bar
2. Dilute 300 µl of whole blood (room temperature) with 300 µl NaCl/$CaCl_2$, preheated at 37°C
3. Incubate diluted blood for 180 sec.
4. Add 20 µl of stock ADPtest reagent.
5. Start test
6. Measure test for 6 min

Note: It is important that the tip of the micropipette is immersed in the sample when the reagent is injected. The saline (NaCl 0.9%) must not contain any additives such as methyl ester. This can cause false-positive results. It is important to pay close attention to temperatures and incubation times. The use of non-preheated NaCl/$CaCl_2$ diluent solution or the introduction of shorter incubation times may skew results.

When using the Multiplate® electronic pipette in auto mode follow the test instructions displayed by the Multiplate® software.

Quality Control

Laboratories should follow generally accepted quality control practices when proficiency testing is not available. It is good laboratory practice to run a drug-free normal control whenever reagents are reconstituted or thawed.

Expected results

Clopidogrel inhibits ADP-induced aggregation. Paniccia et al. investigated 297 patients taking clopidogrel 75 mg / d and reported ADP induced aggregations (10 µM) of 26.8 ± 15.8 U (mean ± SD). The median was 22 U and the range 0-91 U.

Glanzman thrombasthenia leads to reduced ADP induced aggregation. 5 patients with Glanzman thrombasthenia were tested and the following aggregations were determined (median and range): 5 U (1-6 U).

Interpretation of results

Aggregation curves in blood can be interpreted as follows:

- By direct comparison to a normal drug free control that also provides real time quality control
- By comparison to published normal values that can be verified and reproduced by any laboratory

Normal ranges

Note: The following normal range was obtained by analyzing 260 individuals in three centers. It should be used as a guideline only. Normal ranges should be established in each laboratory.

Normal range in whole blood (90% Confidence Interval)		
Reagent	**Concentration**	**AUC (U)**
ADP	10 µM	43-92

Limitations

Samples should be analyzed within the period of 0.5-3 hours after blood collection.

Many drugs inhibit platelet function. Unless the aim of testing is to demonstrate drug-induced inhibition, patients should be drug free for two weeks prior to testing.

A detailed patient history is required for accurate test interpretation. Patients should be questioned about recent ingestion of any medication.

The platelet count in the test sample must be above 100,000 when testing in whole blood.

Literature

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 Dec; 96(6): 781-8.

Paniccia R, Antonucci E, Maggini N, Romano E, Gori AM, Marcucci R, Prisco D, Abbate R. Assessment of platelet function on whole blood by multiple electrode aggregometry in high-risk patients with coronary artery disease receiving antiplatelet therapy. Am J Clin Pathol. 2009 Jun; 131(6): 834-42.

Manufacturer

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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

V.3.0-US-RUO Revised 2011-03

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.

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For in vitro research use only

multiplate®

ASPItest

for platelet aggregation studies

ASPI reagent kit

2°C 8°C

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0110, MP0210, MP0191

V.4.0-US-RUO Revised 2012-02

Product description

The ASPItest reagent is a lyophilized preparation of arachidonic acid (AA), stock concentration 15 mM.

Intended use

The ASPItest reagent is for use in routine platelet aggregation studies for the evaluation of normal platelet function.

Principle

When added to a platelet sample, arachidonic acid triggers platelet activation via a platelet's cyclooxygenase pathway. Arachidonic acid is the substrate of the platelet enzyme cyclooxygenase. Cyclooxygenase transforms arachidonic acid into thromboxane A2, a potent platelet activator.

Materials provided

The reagent is provided in one of three kit formats:

MP0110: ASPItest reagent, 1 x 1.0 ml,
with 5 color coded micro test tubes for aliquotation.

MP0191: ASPItest reagent, 1 x 1.0 ml.

MP0210: ASPItest reagent, 3 x 1.0 ml.

Materials required but not provided

1. Platelet aggregometer
2. Purified water (distilled or deionized)
3. Aggregometer test cells with stir bars
4. Micropipettes – 0.5 µl to 100 µl required for reagents
5. Pipettes – 100 µl to 1 ml required for blood samples
6. Physiological saline (NaCl 0.9%) for irrigation for the dilution of whole blood sample

Instrumentation

The ASPItest reagent will perform as described when used with the Multiplate® Aggregometer. Follow the manufacturer's instructions.

Reagent preparation

Reconstitute each vial of Arachidonic acid reagent 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 is slightly yellow. The staining does not indicate a reduced function.

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

Storage and stability

Lyophilized Reagent: until the expiry date printed on the vial label when stored at 2-8°C.

Reconstituted Reagent: for 24 hours when stored at 2-8°C. When stored at < -20°C the reconstituted reagent is stable for 4 weeks. Reconstituted vials should remain tightly closed when not in use. It is advisable to minimize exposure to light, air and elevated temperatures.

For optimal handling, reconstituted reagent may be aliquoted and the aliquots stored frozen at ≤ -20°C.

Freshly thawed aliquots are stable for 24 hours at room temperature after one freeze thaw cycle.

Warnings and precautions

The ASPItest reagent is for In-vitro-research use only and not for injection or ingestion. Observe standard precautions when handling test specimens and all test materials. Dispose of all waste materials according to local regulations.

Sample collection

Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Collect samples into sterile evacuated tubes with non-wettable lining containing 1/10 volume of 3.2% buffered sodium citrate. Avoid foam formation in the blood collection tube. Always ensure citrated blood collection tubes are filled to the indicated fill volume, in order to avoid excessive citrate levels. Gently invert the collection tube 4 to 5 times to ensure complete mixing of the contents. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

Test procedure

Final working concentration: 0.5 mM AA

1. Insert a disposable test cell with stir bar
2. Dilute 300 µl of whole blood (room temperature) with 300 µl saline (NaCl 0.9%), preheated at 37°C
3. Incubate diluted blood for 180 sec.
4. Add 20 µl of stock ASPItest reagent.
5. Start test
6. Measure test for 6 min

Note: It is important that the tip of the micropipette is immersed in the sample when the reagent is injected. The saline (NaCl 0.9%) must not contain any additives such as methyl ester. This can cause false-positive results. It is important to pay close attention to temperatures and incubation times. The use of non-preheated saline diluent solution or the introduction of shorter incubation times may skew results.

When using the Multiplate® electronic pipette in auto mode follow the test instructions displayed by the Multiplate® software.

Quality Control

Laboratories should follow generally accepted quality control practices when proficiency testing is not available. It is good laboratory practice to run a drug-free normal control whenever reagents are reconstituted or thawed.

Expected results

Aspirin inhibits arachidonic acid-induced aggregation. Mortensen et al. evaluated the arachidonic acid-induced aggregation using Multiplate® (0.5 mM) in 85 diabetics and 92 non-diabetics taking 75 mg aspirin / d and reported aggregations of (median, 25°;75° percentile) 9 U (5 U;14 U) and 7 U (4 U;10 U), respectively.

Glanzman thrombasthenia leads to reduced arachidonic acid-induced aggregation. 5 patients with Glanzman thrombasthenia were tested and the following aggregations were determined (median and range): 3 U (0-20 U).

Interpretation of results

Aggregation curves in blood can be interpreted as follows:

- By direct comparison to a normal drug free control that also provides real time quality control
- By comparison to published normal values that can be verified and reproduced by any laboratory

Normal ranges

Note: The following normal range was obtained by analyzing 260 individuals in three centers. It should be used as a guideline only. Normal ranges should be established in each laboratory.

Normal range in whole blood (90% Confidence Interval)		
Reagent	**Concentration**	**AUC (U)**
Arach. acid	0.5 mM	40-91

Limitations

Samples should be analyzed within the period of 0.5-3 hours after blood collection.

Many drugs inhibit platelet function. Unless the aim of testing is to demonstrate drug-induced inhibition, patients should be drug free for two weeks prior to testing.

A detailed patient history is required for accurate test interpretation. Patients should be questioned about recent ingestion of any medication.

The platelet count in the test sample must be above 100,000 when testing in whole blood.

Literature

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 Dec; 96(6): 781-8.

Mortensen SB, Larsen SB, Grove EL, Kristensen SD, Hvas AM. Reduced platelet response to aspirin in patients with coronary artery disease and type 2 diabetes mellitus. Thromb Res. 2010 Oct; 126(4): e318-22.

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For in vitro research use only

multiplate®

COLtest

for platelet aggregation studies

COL reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0130, MP0230, MP0193

V.4.0-US-RUO Revised 2011-08

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® analyzer[1]. 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 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. 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-$CaCl_2$ 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.

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For in vitro research use only

multiplate®

RISTOtest

for platelet aggregation studies

RISTO reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0140, MP0240, MP0194

V.3.0-US-RUO Revised 2011-03

This box insert is valid for kit formats MP0140, MP0240 and MP0194 of RISTOtest.

Intended use

For in vitro research use only. Reagent for use in platelet aggregation studies on the Multiplate® analyzer[1]. Ristocetin leads to a vWF (von Willebrand factor)- and GpIb-dependent platelet aggregation.

For the evaluation of qualitative platelet dysfunction and disorders of vWF.

Principle

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 RISTOhigh test a high concentration of ristocetin (0.77 mg/ml) is applied, which normally induces a strong platelet aggregation. Abolished aggregation in RISTOhigh test can be based on a deficiency of GpIb receptors or vWF. In RISTOlow test 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 RISTOlow test may indicate an enhanced aggregation tendency of vWF (vWD type IIb).

Reagents

The reagent is provided in three kit formats:

REF MP0140 – RISTOtest: Ristocetin; 1 x 1.0 ml, lyophilised (10 mg/ml), with 5 micro test tubes for aliquotation.

REF MP0194 – RISTOtest: Ristocetin; 1 x 1.0 ml, lyophilised (10 mg/ml), without micro test tubes for aliquotation.

REF MP0240 – RISTOtest: Ristocetin; 3 x 1.0 ml, lyophilised (10 mg/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 (MP0094) for daily use.

Storage and stability

Unopened vials of the RISTOtest 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), 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 RISTOhigh:

300 µl saline 0.9%, preheated at 37°C
+ 300 µl whole blood (hirudin blood / heparin blood / citrated blood, room temperature)
→ 3 minutes incubation
+ **50 µl** RISTOtest reagent
→ Start test → 6 minutes measuring time

Test procedure for RISTOlow:

300 µl saline 0.9%, preheated at 37°C
+ 300 µl whole blood (hirudin blood / heparin blood / citrated blood, room temperature)
→ 3 minutes incubation
+ **12 µl** RISTOtest reagent
→ Start test → 6 minutes measuring time

Final concentrations: 0.77 mg/ml ristocetin (RISTOhigh) or 0.2 mg/ml ristocetin (RISTOlow)

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®.

Note: A partial recalcification of the sample when citrated blood is analyzed (as recommended for the TRAPtest or ADPtest) may lead to disaggregation during the analysis. The reason for this phenomenon is unclear. The use of saline-$CaCl_2$ diluent solution (instead of the use of saline solution) for the analysis of RISTOtest is therefore not recommended.

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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For in vitro research use only

multiplate®

TRAPtest

for platelet aggregation studies

TRAP reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0150, MP0250, MP0195

V.5.0-US-RUO Revised 2012-01

This box insert is valid for kit formats MP0150, MP0250 and MP0195 of TRAPtest.

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 disorders and platelet function inhibition. Sensitive to the action of glycoprotein (Gp) IIb/IIIa receptor antagonists and/or a deficiency of GpIIb/IIIa receptors.

Principle

Thrombin receptor activating peptide-6 (TRAP-6) is a potent platelet activator and stimulates platelet aggregation via the thrombin receptor PAR-1. This leads to a strong platelet activation that may be inhibited by the presence of thrombin receptor antagonists or GpIIb/IIIa receptor antagonists.

Reagents

The reagent is provided in three kit formats:

REF MP0150 – TRAPtest: TRAP-6; 1 x 1.0 ml, lyophilised (1 mM), with 5 micro test tubes for aliquotation.

REF MP0195 – TRAPtest: TRAP-6; 1 x 1.0 ml, lyophilised (1 mM), without micro test tubes for aliquotation.

REF MP0250 – TRAPtest: TRAP-6; 3 x 1.0 ml, lyophilised (1 mM), 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 (MP0095) for daily use.

Storage and stability

Unopened vials of TRAPtest 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. Stable 4 weeks after reconstitution when stored at < -20°C. 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), 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 volume in order to avoid excessive 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 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 TRAPtest 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 TRAPtest reagent
→ Start test → 6 minutes measuring time

Final concentration: 32 µM TRAP-6

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. 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.

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For in vitro research use only

multiplate®

ASA Control

for use as quality control in platelet aggregation studies

ASA reagent kit

Verum Diagnostica GmbH
Munich - Germany

Valid for REF MP0170, MP0270, MP0510

V.4.0-US-RUO Revised 2011-11

This box insert is valid for kit formats MP0170, MP0270 and MP0510 of ASA Control.

Intended use

For in vitro research use only. Reagent for use as quality control in platelet aggregation studies on the Multiplate® analyzer[1,2]. Addition of ASA Control to the blood sample leads to reduced aggregation responses in ASPItest and COLtest.

Principle

ASA Control contains aspirin (30 mg/ml). Upon addition to the blood sample the platelet cyclooxygenase is blocked and therefore cyclooxygenase dependent Multiplate® tests are inhibited, especially ASPItest and COLtest.

This allows the assessment of an abnormal control in these tests.

Reagents

The reagent is provided in three kit formats:

REF MP0170 – ASA Control: acetylsalicylic acid;
1 x 1.0 ml, lyophilised (30 mg/ml), with 5 micro test tubes for aliquotation.

REF MP0510 – ASA Control: acetylsalicylic acid;
1 x 1.0 ml, lyophilised (30 mg/ml), without micro test tubes for aliquotation.

REF MP0270 – ASA Control: acetylsalicylic acid;
3 x 1.0 ml, lyophilised (30 mg/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 (MP0097) for daily use.

Storage and stability

Unopened vials of ASA Control 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 for 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), 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.

COLtest + ASA Control

Test procedure for hirudin or heparin blood:

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

ASPItest + ASA Control

Test procedure:

300 µl saline 0.9%, preheated at 37°C
+ **20 µl** ASA Control
+ 300 µl whole blood (hirudin blood / heparin blood / citrated blood, room temperature)
→ 3 minutes incubation
+ 20 µl ASPItest reagent
→ Start test → 6 minutes measuring time

During incubation time the cyclooxygenase of the platelets in the sample is inhibited by acetylsalicylic acid.

Final concentration: 1 mg/ml acetylsalicylic acid

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] Jámbor C, Weber CF, Gerhardt K, Dietrich W, Spannagl M, Heindl B, Zwissler B. Whole blood multiple electrode aggregometry is a reliable point-of-care test of aspirin-induced platelet dysfunction. Anesth Analg. 2009 Jul; 109(1): 25-31.

[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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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.

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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.

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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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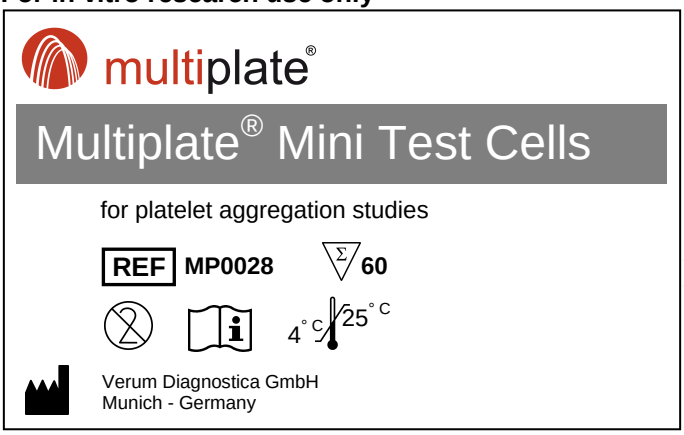

Intended use

For single use in platelet aggregation studies with the Multiplate® platelet function analyzer for low sample volumes of 175 µl whole blood.

Storage and stability

Store the product at 4-25°C. Avoid exposure to air, moisture or direct sunlight. Reseal opened boxes of primary PET packaging accordingly. **Use mini test cells of opened PET boxes within one month after opening.**

Warnings and precautions

For in vitro research use only. Test cells are single use products. Do not reuse. Do not use volumes for analyses below the minimum volume of 350 µl.

General precautions should be followed when handling specimen and all contaminated materials, e.g. wear gloves, minimize exposure of specimen and reagents to the skin. Dispose of all waste materials according to the local regulations.

In the mini test cells during the analysis a smaller blood volume is present compared to the standard test cells. Therefore lower aggregations are found. An analysis with 25 samples of healthy volunteers on average 22% lower aggregations were found using the mini test cells compared to the standard test cells. Therefore reference ranges and target ranges should be determined separately for the mini test cells and standard test cells.

Note: Avoid touching electrode wires when handling new unused test cells and make sure that the stirring bar freely rotates at the bottom of the test cell when inserted in the measuring position.

Performance of the analysis

Follow the instructions in the Multiplate® user manual, short instructions manual and instructions for use for the Multiplate® reagents.

Agonist volumes

For use of the same final concentrations of the standard test assays described in the reagents box inserts please reduce the reagent volumes as follows:

ADPtest / ASPItest / COLtest / TRAPtest:	**12 µl**	RISTOtest high:	**29 µl**
		RISTOtest low:	**7 µl**

Test procedure

The minimum volume of the test cell is 350 µl.

Add 175 µl saline 0,9% (preheated at 37°C)
Add 175 µl whole blood (preferably hirudin or lithium heparin anti-coagulated blood, stored at room temperature)
→ 3 minutes incubation time
Add the appropriate amount of agonist
→ Start test → 6 minutes measuring time

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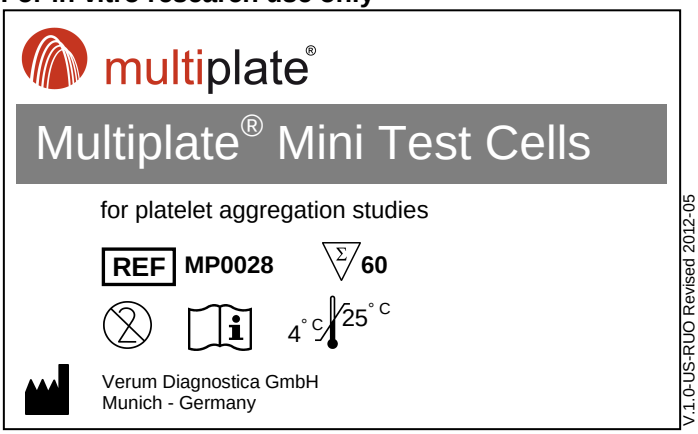

Intended use

For single use in platelet aggregation studies with the Multiplate® platelet function analyzer for low sample volumes of 175 µl whole blood.

Storage and stability

Store the product at 4-25°C. Avoid exposure to air, moisture or direct sunlight. Reseal opened boxes of primary PET packaging accordingly. **Use mini test cells of opened PET boxes within one month after opening.**

Warnings and precautions

For in vitro research use only. Test cells are single use products. Do not reuse. Do not use volumes for analyses below the minimum volume of 350 µl.

General precautions should be followed when handling specimen and all contaminated materials, e.g. wear gloves, minimize exposure of specimen and reagents to the skin. Dispose of all waste materials according to the local regulations.

In the mini test cells during the analysis a smaller blood volume is present compared to the standard test cells. Therefore lower aggregations are found. An analysis with 25 samples of healthy volunteers on average 22% lower aggregations were found using the mini test cells compared to the standard test cells. Therefore reference ranges and target ranges should be determined separately for the mini test cells and standard test cells.

Note: Avoid touching electrode wires when handling new unused test cells and make sure that the stirring bar freely rotates at the bottom of the test cell when inserted in the measuring position.

Performance of the analysis

Follow the instructions in the Multiplate® user manual, short instructions manual and instructions for use for the Multiplate® reagents.

Agonist volumes

For use of the same final concentrations of the standard test assays described in the reagents box inserts please reduce the reagent volumes as follows:

ADPtest / ASPItest / COLtest / TRAPtest:	**12 µl**	RISTOtest high:	**29 µl**
		RISTOtest low:	**7 µl**

Test procedure

The minimum volume of the test cell is 350 µl.

Add 175 µl saline 0,9% (preheated at 37°C)
Add 175 µl whole blood (preferably hirudin or lithium heparin anti-coagulated blood, stored at room temperature)
→ 3 minutes incubation time
Add the appropriate amount of agonist
→ Start test → 6 minutes measuring time

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