GpIIb/IIIa Antagonist Reagent

GpIIb/IIIa Antagonist Reagent

REF		CONTENT	SYSTEM
08847568190	08847568500	3 x 0.5 mL	Multiplate

English

Intended use

The reagent is intended for use as quality control in platelet function testing with whole blood samples on the Multiplate analyzer. The reagent is employed in combination with the Multiplate activating reagent TRAPtest. Addition of GpIIb/IIIa Antagonist Reagent to a blood sample leads to strongly reduced aggregation in the TRAPtest.

Summary

In vitro platelet aggregation by electrical impedance relies on recruitment of platelets to a surface (electrode wire) by means of platelet-fibrinogen bonds in order to measure a response. When platelets are activated, the individual components of the glycoprotein IIb/IIIa receptors (GpIIb/IIIa) on the platelet membrane physically alter their conformation producing a high-affinity fibrinogen binding site. The introduction of a substance that blocks the binding of fibrinogen to the resting or activated GpIIb/IIIa receptor will result in reduced or absent platelet aggregation to all agonists including TRAP-6.[1,2]

Test principle

The GpIIb/IIIa Antagonist Reagent 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 diminished aggregation in Multiplate tests. This allows the assessment of a positive control (strongly inhibited aggregation).[1]

In the Test Cells activated platelets adhere to and aggregate on the sensor wires. This leads to an increased resistance between the sensor wires, which is continuously recorded and expressed via the area under the curve (AUC) in arbitrary units (AU*min or U; conversion: 1 U = 10 AU*min).[3]

Reagents - working solutions

R1[a]: Liquid reagent containing synthetic GpIIb/IIIa antagonist: molecular weight 495 g/mol in a concentration of 50 µg/mL. 3 vials, each containing 0.5 mL

a) Reagent 1

Precautions and warnings

For in vitro diagnostic use for laboratory professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagent is ready for use.

Storage and stability

Store at 2-8 °C.

The reagents are stable up to the stated expiration date. Store the vials in an upright position.

Stability of the reagent after opening:	
at 15-25 °C	24 hours
at 2-8 °C	28 days

Protect reagent from exposure to light, air and elevated temperature ranges.

Specimen collection and preparation

Only the specimen listed below was tested and found acceptable: venous whole blood.

Use standard sampling tubes to collect the sample and follow the instructions for use from the appropriate manufacturer. Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Avoid foam formation in the blood collection tube.

Gently invert the collection tube to ensure complete mixing of the contents.[4]

Do not store the blood sample on a roll mixer.

Do not freeze or refrigerate samples. Do not preheat the blood before analysis.[5,6]

The anticoagulant used for blood sample collection significantly affects the results of the assay.[5,7] The use of commercial hirudin blood collection tubes is recommended.[8]

Alternatively standard citrated tubes (3.2 % citrate) may be used.[9,10,11] Always ensure citrated blood collection tubes are filled to the indicated fill volume in order to avoid excessive citrate levels.

The blood collection system must be standardized at each center. It is only possible to compare the results of an individual sample with reference ranges when the same sample anticoagulant is employed. Sample collection systems from various manufacturers may contain differing materials which could affect the test results. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Visually check the blood samples for clots before measurement. If clots are found the blood sample has to be refused. Microthrombi in the sample could adversely affect the test results. Mix the blood sample by inverting the tube gently just before measurement.

Materials provided

- See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 06675972190, NaCl/$CaCl_2$ Solution

GpIIb/IIIa Antagonist Reagent

GpIIb/IIIa Antagonist Reagent

- REF 08847509190, TRAPtest, 3 x 1.0 mL
- REF 06675590001, Test Cells, 6 x 10 pieces
- Saline solution 0.9 % (without additives, e.g. methyl ester)
- Multiplate analyzer. See operator's manual and Quick Start Guide of the analyzer for additionally required materials.
- Distilled or deionized water
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Ensure to incubate the GpIIb/IIIa Antagonist Reagent with the sample before adding the agonist.

Place R1, SR and Test Cells in the designated positions.

Test procedure for hirudinized blood:	
Saline solution 0.9 % (prewarmed to 37 °C)	300 µL
R1	20 µL
Sample (15-25 °C)	300 µL
Incubation	180 seconds
TRAPtest (SR[b)])	20 µL
Measuring time	6 minutes

b) Start reagent

Test procedure for citrated blood:	
NaCl/$CaCl_2$ Solution (prewarmed to 37 °C)	300 µL
R1	20 µL
Sample (15-25 °C)	300 µL
Incubation	180 seconds
TRAPtest (SR)	20 µL
Measuring time	6 minutes

Final concentration: 1.6 µg/mL GpIIb/IIIa antagonist

Temperature conditions and incubation times must be precisely observed. When using the electronic pipette follow the software instructions displayed by the Multiplate analyzer.

Quality control

A normal blood sample can be used as a control of the activity and stability of the reagent.

Follow the applicable government regulations and local guidelines for quality control.

Expected values

The GpIIb/IIIa Antagonist Reagent serves as an antagonist and expected values have not been established.

Specific performance data

Representative performance data on the Multiplate analyzer is given below. Results obtained in individual laboratories may differ.

Inhibition

Inhibition was determined by measuring the TRAPtest with and without the addition of the GpIIb/IIIa Antagonist Reagent into a hirudinized whole blood sample. Measurements were performed on 2 devices on all channels (i.e. 10 measurements with and without the antagonist). The mean result before and after adding the antagonist and the final percent inhibition were calculated. Testing was performed on a total of 10 blood samples using the same devices and the same vials of reagent per sample.

The following results were obtained:

Blood sample	Mean value [U] TRAPtest without GpIIb/IIIa (n = 5)	Mean value [U] TRAPtest with GpIIb/IIIa (n = 5)	Inhibition of TRAPtest [%]
1	137	0	100
2	155	0	100
3	101	0	100
4	125	0	100
5	131	0	100
6	96	0	100
7	116	0	100
8	88	2	98
9	126	0	100
10	110	0	100

References

1 Desch S, Siegemund A, Scholz U, et al. Platelet inhibition and GP IIb/IIIa receptor occupancy by intracoronary versus intravenous bolus administration of abciximab in patients with ST-elevation myocardial infarction. Clin Res Cardiol 2011 Oct 21.

2 Penz S, Perchuc A, Calatzis A, et al. Effects of GpIIbIIIa and GpIb blockade on multiple electrode aggregometry (MEA). GTH 2010; Abstract P10-13.

3 Sibbing D, Braun S, Jawansky S, et al. Assessment of ADP-induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidogrel treatment. Thromb Haemost 2008; 99: 121-126.

4 CLSI. Platelet Function Testing by Aggregometry; Approved Guideline. CLSI document H58-A. Clinical and Laboratory Standards Institute; 2008.

5 Kaiser AF, Neubauer H, Franken CC, et al. Which is the best anticoagulant for whole blood aggregometry platelet function testing? Comparison of six anticoagulants and diverse storage conditions. Platelets. 2011 Oct 14.

6 Sweeney JD, Hoernig LA, Michnik A et al. Whole blood aggregometry. Influence of sample collection and delay in study performance on test results. Am J Clin Pathol. 1989 Nov;92(5):676-9.

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

8 Tóth O, Calatzis A, Penz S et al. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. Thromb Haemost. 2006 Dec;96(6):781-8.

9 Jennings LK, White MM. Platelet aggregation. In: Michelson AD, ed. Platelets. 2nd ed. Amsterdam: Elsevier; 2007:495-518.

10 Velik-Salchner C, Maier S, Innerhofer P et al. Point-of-care whole blood impedance aggregometry versus classical light transmission aggregometry for detecting acetylsalicylic acid and clopidogrel: the results of a pilot study. Anesth Analg. 2008 Dec;107(6):1798-806.

11 Paniccia R, Antonucci E, Maggini N et al. 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.

For further information, please refer to the appropriate operator's manual and quick start guide for the Multiplate analyzer, the respective applications sheets, the product information, and Method Sheets of all necessary components.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

GpIIb/IIIa Antagonist Reagent

GpIIb/IIIa Antagonist Reagent

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606