TRAPtest

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REF		CONTENT	SYSTEM
08847509190	08847509500	3 x ⟶ 1.0 mL	Multiplate

English

Intended use

Assay for the quantitative in vitro determination of platelet function triggered by TRAP-6. The reagent is intended for use in platelet function testing with whole blood samples on the Multiplate analyzer.

Summary

Thrombin is capable of activating platelets, which is mediated primarily by the hydrolysis of a G-protein-coupled receptor on the platelet membrane, referred to as protease-activated receptor 1 (PAR1) and a second receptor (PAR4) that expresses a lower sensitivity to thrombin.[1] Activation by thrombin results in cross linked platelet aggregation as fibrinogen strands bind to glycoprotein IIb/IIIa receptors (GpIIb/IIIa) on the platelet membrane. Upon activation the components of the GpIIb/IIIa receptors physically alter their conformation producing a high-affinity fibrinogen binding site. In order to analyze platelet function triggered via the thrombin receptor, a peptide which stimulates the PAR1 receptor is commonly used (SFLLRN = TRAP-6).[2] This allows to test for platelet function activated by the PAR1 receptor, without triggering fibrin formation in the sample, which would happen if thrombin was used as the agonist.

TRAP-6-induced platelet aggregation may be reduced or absent in the presence of GpIIb/IIIa receptor antagonists[3] or in deficiency states of GpIIb/IIIa receptors (Glanzmann thrombasthenia)[4,5] and PAR1 inhibitor, Vorapaxar.[6] TRAP-6-induced aggregation displays a minor sensitivity for the inhibiting effects of acetylsalicylic acid[7,8,9] or ADP receptor antagonists.[10,11,12]

Test principle

Thrombin receptor activating peptide 6 (TRAP-6) is a potent platelet activator and stimulates platelet aggregation via the thrombin receptor PAR1. This leads to a strong platelet activation.

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).[13]

Reagents - working solutions

- **SR**[a]: Lyophilized reagent containing TRAP-6: 1 mmol/L; 3 vials, each for 1.0 mL

a) Start reagent

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

H315 Causes skin irritation.

H317 May cause an allergic skin reaction.

H319 Causes serious eye irritation.

H411 Toxic to aquatic life with long lasting effects.

Prevention:

P261 Avoid breathing dust.

P264 Wash skin thoroughly after handling.

P273 Avoid release to the environment.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

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

P391 Collect spillage.

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

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

Hazardous components:

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

Reagent handling

Carefully reconstitute the contents of one vial of R1 by adding 1.0 mL of distilled or deionized water. Gently swirl R1 and allow vial to stand closed for 10 minutes at 18-25 °C. Swirl the vial carefully to produce a homogeneous solution before use. Avoid the formation of foam.

Note: The vials are filled with an inert gas instead of a vacuum. After reconstitution you can pipette ≥ 100 µL aliquots of the reagent into micro test tubes for daily use.

The solution is slightly yellow. The color does not indicate a reduction in function.

Storage and stability

Store at 2-8 °C.

The lyophilized reagents are stable up to the stated expiration date. Store the reconstituted reagent (aliquoted or in the original vial) in an upright position.

Stability of the reconstituted reagent either:	
at 15-25 °C	24 hours
at 2-8 °C	7 days
at -20 °C (±5 °C)	28 days
after 1 time thawing at 15-25 °C	24 hours

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

Do not re-freeze.

Thaw the reagent at 15-25 °C for at least 1 hour while kept in an upright position. Avoid the contact of the reagent with the rubber stopper. Swirl gently the original vial or micro test tube to homogenize the solution prior to use.

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.[14]

Do not store the blood sample on a roll mixer.

Do not freeze or refrigerate samples. Do not preheat the blood before analysis.[15,16]

The anticoagulant used for blood sample collection significantly affects the results of the assay.[15,17] The use of commercial hirudin blood collection tubes is recommended.[18]

Alternatively standard citrated tubes (3.2 % citrate) may be used.[19,20,21] 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 (i.e. lithium-heparin, citrate or huridin) is employed.

Analyze blood samples within the period of 0.5 to 3 hours after blood collection.

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
- REF 08847568190, GpIIb/IIIa Antagonist Reagent, 3 x 0.5 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.

Place SR and Test Cells in the designated positions.

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

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

Final concentration: 32 µmol/L TRAP-6

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.

An abnormal inhibited aggregation can be achieved by addition of GpIIb/IIIa Antagonist Reagent.[13]

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

Limitations - interferences

Impaired platelet function has been reported after the ingestion of various drugs and herbal medicines.[22,23] Aggregation may be abnormal in patients with thrombocytopenia.[24,25]

The ingestion of acetylsalicylic acid and[10,11,12,23] ADP-receptor antagonists may lead to weak aggregation.

Certain fatty acids found in various human diets are widely known to inhibit platelet function.[26,27]

No significant effect on the test result has been observed with hematocrit values between 34 to 55 %.

Hemolysis may interfere with test results. Therefore, the use of hemolyzed blood samples is not recommended.

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested.

Endogenous substances: No impact on results was observed up to the listed concentrations.

Substance	**Concentration**
Conjugated bilirubin	1 mg/dL
Unconjugated bilirubin	33 mg/dL

Pharmaceutical compounds: Interference was observed at listed therapeutic concentrations.

Compound	**Drug category**	**Therapeutic concentration**	**Effect on TRAPtest**
Dextran 40	Replacement agent	20 g/L	Decrease
Ethanol	Local anesthetic	43.4 mmol/L	Decrease
Hydroxyethyl starch	Replacement agent	45.3 g/L	Decrease
Penicillin G	β-lactam antibiotic	10000 IU/mL	Decrease
Streptokinase	Thrombolytic agent	100000 IU/L	Decrease
Ticagrelor	Anticoagulant	27.7 mg/L	Decrease
LMWH (Heparin)	Anticoagulant	1000 IU/L	Increase

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Expected values

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Expected values have been established in a study with 156 healthy donors using Sarstedt S-Monovette 1.6 mL hirudin tubes.
Result (2.5^{th} to 97.5^{th} percentile):

Tube type	**AUC [U]**
Hirudin tubes	97-182

Expected values have been established in a study with 157 healthy donors using BD Vacutainer 9NC 0.109 M Buff. Na_3 Citrate Tubes.

Result (2.5^{th} to 97.5^{th} percentile):

Tube type	**AUC [U]**

Citrated tubes (buffered)	86-159

Note: For establishing reference values the normal donor must not take nonsteroidal anti-inflammatory drugs (NSAIDs) or ADP-receptor antagonists within 10 days prior to testing.

Specific performance data

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

Precision

Repeatability was determined for 3 reagent lots by measuring 12 hirudinized blood samples on 6 devices on all channels with 2 runs (i.e. 360 measurements in total). Mean, coefficient of variation (CV) and standard deviation (SD) were calculated for every blood sample. The pooled mean, pooled coefficient of variation (CV_{pool}) and pooled standard deviation (SD_{pool}) were calculated for every reagent lot.

TRAPtest reagent lot	n (native)	$Mean_{pool}$ [U]	CV_{pool} [%]
1	360	125	7
2	360	125	6
3	360	126	6

TRAPtest reagent lot	n (low)	$Mean_{pool}$ [U]	SD_{pool} [U]
1	360	45	4
2	360	39	7
3	360	39	3

References

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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.

Symbols

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

TRAPtest

TRAPtest

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

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