

ADPtest

Roche

ADPtest

REF	CONTENT	SYSTEM
08847550190	08847550500	3 x → 1.0 mL
		Multiplate

English

Intended use

Assay for the quantitative in vitro determination of platelet function following stimulation of the platelet adenosine diphosphate (ADP) receptors. The reagent is intended for use in platelet aggregation testing with whole blood samples on the Multiplate analyzer.

Summary

Adenosine diphosphate (ADP) is a nucleotide that is stored in the dense granules of platelets. Upon activation, platelets undergo shape change and release the contents of their alpha and dense granules. The release of internal stores of ADP from the dense granules serves to further activate adhered platelets in addition to activating local free circulating platelets by binding to purinergic receptors, P_2Y_1 and P_2Y_{12} on platelet membranes. 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. Fibrinogen, both freely circulating and released from the alpha granules, subsequently binds to the GpIIb/IIIa receptors forming platelet-to-platelet bridges resulting in platelet aggregation.¹ ADP-induced platelet aggregation may be reduced or absent in the case of Glanzmann's thrombasthenia or individuals ingesting thienopyridines.^{2,3,4} Because platelet aggregation ultimately relies on the formation of GpIIb/IIIa – fibrinogen bridges, GpIIb/IIIa antagonists may reduce or eliminate an ADP-induced aggregation.⁵

Test principle

The ADPtest reagent contains ADP, which triggers platelet activation via platelet ADP receptors. 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).⁴ The ADPtest assay is sensitive to an inhibition of the P_2Y_{12} receptor^{4,6} as well as an inhibition or absence of the GpIIb/IIIa receptor.^{2,5,7}

Reagents - working solutions

- SR^{a)}: Lyophilized reagent containing ADP: 0.2 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. |
|------|-------------------|

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

Carefully reconstitute the contents of 1 vial of SR by adding 1.0 mL of distilled or deionized water. Gently swirl SR and allow vial to stand closed for a minimum of 10 minutes at 15-25 °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 should be clear and colorless.

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.⁸

Do not store the blood sample on a roll mixer.

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Do not freeze or refrigerate samples. Do not preheat the blood before analysis.^{9,10}

The anticoagulant used for blood sample collection significantly affects the results of the assay.^{9,11} The use of commercial hirudin blood collection tubes is recommended.¹²

The widest experience for the monitoring of ADP-receptor antagonists currently exists for hirudin blood.^{13,14,15}

Alternatively standard citrated tubes (3.2 % citrate) may be used.^{16,17,18} 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.

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

Analyze citrated blood samples within the period of 1 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/CaCl₂ Solution
- [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 for the analyzer concerned. 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: 6.5 µmol/L ADP

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.⁴

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

Limitations - interference

Impaired platelet function has been reported after the ingestion of various drugs and herbal medicines.^{19,20} Aggregation may be abnormal in patients with thrombocytopenia.^{21,22}

The presence of GpIIb/IIIa antagonists in the sample leads to weak aggregation.⁴

Certain fatty acids found in various human diets are widely known to inhibit platelet function.^{23,24}

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 ADPtest
N-Acetylcysteine (ACC)	Mucolytic agent	3.4 mmol/L	Decrease
Dabigatran	Anticoagulant	17.5 mg/dL	Decrease
Dextran 40	Replacement agent	20 g/L	Decrease
Dipyridamole	Antiplatelet drug	19.8 µmol/L	Decrease
Ethanol	Local anesthetic	43.4 mmol/L	Decrease
Hydroxyethyl starch	Replacement agent	45.3 g/L	Decrease
Meloxicam	Nonsteroidal anti-inflammatory drug	2.5 µg/mL	Decrease
Penicillin G	β-lactam antibiotic	10000 IU/mL	Decrease
Streptokinase	Thrombolytic agent	100000 IU/L	Decrease

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.

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Result (2.5th to 97.5th percentile):

Tube type	AUC [U]
Hirudin tubes	53-140

Expected values have been established in a study with 157 healthy donors using BD Vacutainer 9NC 0.109 M Buff. Na₃ Citrate Tubes.

Result (2.5th to 97.5th percentile):

Tube type	AUC [U]
Citrated tubes (buffered)	48-119

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.

ADPtest reagent lot	n (native)	Mean _{pool} [U]	CV _{pool} [%]
1	360	96	10
2	360	90	7
3	360	83	8

ADPtest reagent lot	n (low)	Mean _{pool} [U]	SD _{pool} [U]
1	360	26	4
2	360	41	4
3	360	26	4

Method comparison

A comparison of the ADPtest on a Multiplate analyzer with an automated platelet function test gave the following conformity between results in the normal and in the low range:

Number of samples measured: 114

Concordance (%): 87

Results of the ADPtest were between 7 U and 124 U.

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.

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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

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Additions, deletions or changes are indicated by a change bar in the margin.

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