Prostaglandin E1 Reagent

Prostaglandin E1 Reagent

REF		CONTENT	SYSTEM
08847525190	08847525500	3 x ⟶ 1.0 mL	Multiplate

English

Intended use

Reagent for the quantitative in vitro determination of platelet function triggered by adenosine diphosphate and Prostaglandin E1 Reagent (in conjunction with reagent ADPtest). The reagent is intended for use in platelet function testing with whole blood samples on the Multiplate analyzer. For the assessment of ADPtest HS (high sensitivity).

Summary

Adenosine diphosphate (ADP) induced platelet aggregation is achieved through binding of the ADP molecule to purinergic receptors, P_2Y_1 and P_2Y_{12}, on platelet membranes. The P_2Y_1 receptor is associated with the mobilization of calcium stores and the initiation of ADP-induced aggregation. Stimulation of the P_2Y_1 receptor causes an inhibition of cyclic adenosine monophosphate (cAMP), thus facilitating the activation process.

The P_2Y_{12} receptor, the more important one of the two receptors, is attributed with the amplification and completion of the aggregation process. The P_2Y_{12} receptor is also responsible for the sustained aggregation effect of ADP stimulation.[1,2]

Prostaglandin E1 Reagent (PGE1) increases cAMP concentrations by stimulating adenyl cyclase activity in platelets. The increase in cAMP inhibits calcium mobilization and platelet aggregation induced by P_2Y_1 receptor activation. The addition of PGE1 to a blood sample can reduce the platelet activation by the P_2Y_1 receptor in a concentration dependent manner. This effect enables the more sensitive evaluation of substances that affect platelet aggregation through P_2Y_{12} receptor binding.[3,4]

Test principle

PGE1 is used in combination with the ADPtest reagent. The addition of 20 µL PGE1 to the ADPtest 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. Therefore this modified assay is named ADPtest high sensitivity (HS).[3,5]

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).[6]

Reagents - working solutions

- **R1**[a]: Lyophilized reagent containing prostaglandin E1: 300 nmol/L; 3 vials, each for 1.0 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

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 R1 by adding 1.0 mL of distilled or deionized water. Gently swirl R1 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

Prostaglandin E1 Reagent

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.[7]

Do not store the blood sample on a roll mixer.

Do not freeze or refrigerate samples. Do not preheat the blood before analysis.[8,9]

The anticoagulant used for blood sample collection significantly affects the results of the assay.[8,10] The use of commercial hirudin blood collection tubes is recommended.[11]

There is no experience for this reagent with the use of citrated blood.

The blood collection system must be standardized at each center. 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 1 hour 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 08847550190, ADPtest, 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.

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

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

b) Start reagent

Final concentration: 9.4 nmol/L PGE1

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.

Limitations - interference

Impaired platelet function has been reported after the ingestion of various drugs and herbal medicines.[12,13] Aggregation may be abnormal in patients with thrombocytopenia.[14,15]

The presence of ADP-receptor antagonists in the sample may lead to weak aggregation.[16,17]

Certain fatty acids found in various human diets are widely known to inhibit platelet function.[18,19]

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 HS**
N-Acetylcysteine (ACC)	Mucolytic agent	3.4 mmol/L	Decrease
Apixaban	Anticoagulant	10.5 µg/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
LMWH (Heparin)	Anticoagulant	1000 IU/L	Increase
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 154 healthy donors using Sarstedt S-Monovette 1.6 mL hirudin tubes.
Result (2.5th to 97.5th percentile):

Tube type	**AUC [U]**
Hirudin tubes	38-141

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

Prostaglandin E1 Reagent

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.

Prostaglandin E1 Reagent lot	n (native)	$Mean_{pool}$ [U]	CV_{pool} [%]
1	360	92	6
2	360	78	8
3	360	63	12

Prostaglandin E1 Reagent lot	n (low)	$Mean_{pool}$ [U]	SD_{pool} [U]
1	360	14	4
2	360	24	3
3	360	19	3

Method comparison

A comparison of the ADPtest HS 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 (%): 91

Results of the ADPtest HS using the Prostaglandin E1 Reagent were between 2 U and 120 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.

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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

+800 5505 6606