CE
In Vitro
Diagnostic

Behnk Elektronik

BE LTA Epi 100 Epinephrine

Reagent for the determination of light transmission aggregometry in platelet rich plasma

REF 057631: RE (2 x 1 mL), DIL (1 x 20 mL)

INTENDED USE

This reagent is designated for professional in vitro diagnostics in the laboratory. It is used for the analysis of platelet function based on light transmission aggregometry (LTA) in platelet-rich human plasma.
The test assists in the diagnosis of platelet dysfunction and can be used as an aid in the management of patients with known dysfunction. Examination and evaluation should always include multiple agonists, which are considered in context.
This test should be used in conjunction with other clinical and diagnostic information to diagnose and treat patients.

PRINCIPLE

Platelet rich plasma (PRP) is mixed with Epinephrine (**Epi**). Platelet aggregation is measured under constant mixing. A change in light transmission occurs. Evaluated are the time from adding the reagent until the onset of the shape change or the aggregation, respectively, the velocity of aggregation (slope) and the maximum aggregation (in percent). See the manual or the literature for further details.

CLINICAL SIGNIFICANCE

Epinephrine (**Epi**) activates the G-protein coupled α2-adreno receptor. This induces direct aggregation without "shape change" by exposure of the fibrinogen receptors, release of calcium ions from the endoplasmatic reticulum and inhibition of adenylate cyclase, inducing a reversible formation of aggregates. A second aggregation wave can be induced by release of ADP and thromboxane A2 from platelet granules. But this does not occur in all cases.
Healthy persons often show a weak reaction on **Epi**, specifically the absence of a second wave. But also hyper reactivity is found (4), probably by genetic causes. (5), (6)
Defects of the platelet α2-adreno receptor may lead to bleeding. (7) In other congenital platelet function defects, the reactivity against Epi may be heterogeneous. Abnormal results can be found often in Bernard-Soulier syndrome, in storage pool defect, and in thrombasthenia Glanzmann, though not in all cases. Also reactivity after aspirin ingestion may be heterogeneous.

REAGENTS

RE **Epi** **100 µM**

Epinephrine ((R)-1-(3,4-Dihydroxyphenyl)-2-(N-methylamino)-ethanol), as bitartrat, stabilizers.

DIL **LTA Diluent**

Dilution buffer

Additional content:
Silicone Caps (Prevent evaporation)
Barcodes for the Thrombomate®

SAFETY CAUTIONS

Behnk reagents are designated for professional in-vitro-diagnostic use.
Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples and should be handled as potentially infectious.
For further information, Material Safety datasheet is available upon request.
Dispose of waste in accordance with the local regulations.

PREPARATION OF REAGENTS

RE: Remove the screw cap. First carefully lift the stopper of the bottle to release the bottle vacuum caused by production, then remove the stopper.
Reconstitute the lyophilizate with exactly 1 ml LTA Diluent. Allow to stand for 10 min, then swirl carefully.

Fixing the **silicone caps** when using the reagent on the Thrombomate:
Lock each reagent vial and the Clean Pro bottle with a silicone cap. Carefully swirl the reagent in a circular motion before each insertion into the device.

Notice: The silicone caps should remain on the bottles for their entire lifetime (even in the refrigerator). They are pierced by the pipetting needle during operation.

DIL: Ready to use.

STABILITY AND STORAGE

Storage at 2-8 °C.
The un-opened reagents are stable until the declared expiration date.

Storage after reconstitution
The reconstituted reagent shall be stored capped with the silicon cap at 2-8 °C in the original vial. Do not freeze.

Stability after reconstitution
Reagent is stable in the original vial with the silicone cap:

- At 2-8 °C 28 days
- At 15-25 °C 14 days
- Laboratory mode* 21 days

** Laboratory mode* = 8 h on board, 16 h 2-8 °C

Notice: If the reagent is not used for a longer period of time, it is recommended to store the reagent with silicone cap closed at 2-8 °C in the original vial.

With the many different combinations of storage conditions, it is recommended that each laboratory observe the stability of the reagent, based on its own usage behaviour. The times determined above are determined under the specified conditions and must not be exceeded.

Notice: After the specified stability has expired, the reagent must no longer be used. In this case, the reagent will not be accepted by the Thrombomate.

SAMPLES COLLECTION AND HANDLING

Blood collection for aggregation testing should be made as gentle as possible into commercially available collection tubes. Use 0.11 M sodium citrate for anticoagulation.
Blood collection should be made by very gentle venous puncture, preferably without or with just minimum stasis of the vein. Mix blood and anticoagulant well by gentle inverting. Avoid foam formation. Store the sample at 15-25 °C. Do not expose the blood to lower temperatures than 15 °C and avoid mechanical stress through shaking during transportation and storage because this may lead to platelet activation. Pneumatic transportation systems are not recommended unless carefully validated.

Sample stability:
Maximum 4 hours after blood collection until completed analysis.

For **preparing platelet rich plasma (PRP)** the blood is centrifuged at room temperature for 150 x g. Optionally centrifuge again for 5 min (at 150 g) if there are erythrocytes visible in the supernatant.
Notice: Switch off the automatic brake function.
For better standardization and comparability of results use always the same centrifuge.
PRP is gently transferred with a disposable plastic pipette or a pipette with disposable plastic tip into a Thrombomate sample tube. Close this with the red cap piercing stopper.
Let the PRP rest for 30 min prior to analysis. The platelet count should be determined because a certain minimum quantity of platelets is required for a reliable measurement.

Adjustment of the platelet count by mixing of PRP with autologous platelet free plasma is not longer recommended, except in extremely low platelet numbers. (1),(2)
For **preparation of platelet free plasma (PPP)** the residual blood of the sample or from a different sample of the patient is centrifuged for 20 min at 1500x g. Transfer the supernatant gently into a different sample tube.
Notice: Avoid formation of foam. Do not invert or place the tube into a horizontal position because this may induce bubble formation.

LIMITS

Several **preanalytical factors** (conditions of venous stasis, puncture of the vein, canula, anticoagulant, type of the tube, type of tub, conditions for sample transportation, residual number of erythrocytes, resting time after venepucture and others) may cause variable deviations.Therefore each lab should determine their own reference ranges.
For interpretation of results in **patient samples** results obtained with other lab tests should be considered (for example von Willebrand Factor, fibrinogen, whole blood count, aggregometry with other reagents or concentrations).
Many dietetic factors or drugs may influence platelet function (3). See the literature for further information.
Lipemia, bilirubin or hemolysis may influence results. Results may also be influenced by the platelet count. Platelet counts < 75/nl may lead to lower results.

MATERIAL REQUIRED BUT NOT PROVIDED

- *BE Thrombomate® XRA* or Manual device for measuring light transmission aggregometry.
- General equipment for a medical laboratory
- *Sample tubes* (REF 057400)
- *BE Clean Pro* (REF 050951)
- *BE LTA Cuvette* Set (1000 Cuvettes) (REF 057600)
- *BE X-Tray* (REF 691041; REF 691042; REF 691043; REF 691044)

PROCEDURE

Automatic method on Behnk Thrombomate®
Running a test is fully automated after reagents and samples have been inserted into the instrument. See the Thrombomate® manual for further details.

Manual method
The test is performed according to the specifications of the various instrument manufacturers.

CALIBRATION

Not required

CALCULATION

See device manufacturer.

QUALITY CONTROL

For quality control it is recommended to analyze with each series of patient samples also a sample from a known healthy donor without medication. This sample should be processed and analyzed like the patient samples.

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PERFORMANCES

Precision was determined on the Thrombomate® XRA compared with PAP-8 (manual system) for the maximum value of aggregation (%).
Analysis of precision was performed by 5-fold analysis of PRP from 5 different individuals.
Notice: All measurements with PAP-8 were performed by the same operator.

		Thrombomate® XRA	Manual System
Reagent	Test concentration	Mean CV	Mean CV
Epi	5.0 µM	**2.2**	**2.8**

Table 1: Precision analysis (5-fold determination, 5 individuals).
Numbers represent the CV values in %.

EXPECTED VALUES

Notice: Each laboratory should establish its own normal range for each agonist.
Expected results of healthy subject:

Reagent	**Concentration**	**% Aggregation**
Epi	5 µM	>70 %

General aspects:

REFERENCES

(1) *Linnemann B, et al. Standardization of light transmittance aggregometry for monitoring antiplatelet therapy: an adjustment for platelet count is not necessary. J Thromb Haemost. 2008;6:677-83*
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(3) *Bachmair EM, Ostertag LM, Zhang X, de Roos B. Dietary manipulation of platelet function. Pharmacol Ther. 2014; 144:97-113.*
(4) *Berger JS, Becker RC, Kuhn C, Helms MJ, Ortel TL, Williams R. Hyperreactive platelet phenotypes: relationship to altered serotonin transporter number, transport kinetics and intrinsic response to adrenergic co-stimulation. Thromb Haemost. 2013;109:85-92*
(5) *Peace AJ, Mangiacapra F, et al. a2A-Adrenergic receptor polymorphism potentiates platelet reactivity in patients with stable coronary artery disease carrying the cytochrome P450 2C19*2 genetic variant. Arterioscler Thromb Vasc Biol. 2014; 34: 1314-9.*
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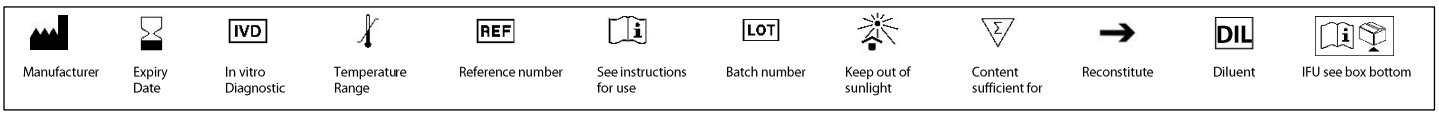
(7) *Rao AK, Willis J, Kowalska MA, et al. Differential requirements for platelet aggregation and inhibition of adenylate cyclase by epinephrine: studies of a familial platelet alpha 2-adrenergic receptor defect. Blood. 1988; 71: 494-501.*



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