

BE LTA Ris 15 Ristocetin

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

REF 057651: 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 Ristocetin (**Ris**). Platelet aggregation (or agglutination in the case of **Ris**) 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

Ristocetin (**Ris**), an antibiotic from *Nocardia lucida*, induces as a cofactor of VWF (von Willebrand-Factor) aggregation (or agglutination) of platelets via the VWF receptor (GP1b-V-IX complex) in the platelet membrane.

In patients with VWF syndrome, the following results are found in many but not in all cases:

VWF deficiency type	Agglutination
Type 1	No or lowered
Type 2A	No or lowered
Type 2B	Enhanced
Type 3	No or very low

In patients with Bernard-Soulier syndrome there is no aggregation/ agglutination, or it is drastically reduced. In Storage-Pool disease and thrombasthenia Glanzmann the first phase of agglutination is frequently affected (1)(2)(3). Defects of cyclooxygenase or thromboxane synthase or of aspirin are not detected. To some extent, results depend on platelet counts.

REAGENTS

RE **Ris** **15 mg/ml**

Ristocetin, an antibiotic that agglutinates platelets in the presence of von Willebrand-Factor.

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. (4),(5)

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 venepuncture 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 (6). 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.

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.

Reagent	Test concentration	Thrombomate® XRA	Manual System
		Mean CV	Mean CV
Ris	1.2 mg/ml	2.1	1.7

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
Ris	1,2 mg/ml	>80 %
	0,6 mg/ml	<15 %

REFERENCES

- (1) U Budde et al. Standardisierte Diagnostik des von Willebrand- Syndroms. *Hämostaseologie* 24, 12 (2004)
- (2) M. Franchini, *Advances in the diagnosis and management of von Willebrand disease. Hematology.* 11, 219 (2006)
- (3) Franchini M. Acquired von Willebrand Syndrome: An Update. *American Journal of Hematology* 82:368-375 (2007)
- (4) 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
- (5) Cattaneo M, et al. Platelet aggregation studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize platelet count. 2007;92: 694-7.
- (6) Bachmair EM, Ostertag LM, Zhang X, de Roos B. Dietary manipulation of platelet function. *Pharmacol Ther.* 2014; 144:97-113.

											
Manufacturer	Expiry Date	In vitro Diagnostic	Temperature Range	Reference number	See instructions for use	Batch number	Keep out of sunlight	Content sufficient for	Reconstitute	Diluent	IFU see box bottom

Manufacturer:
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