COLLAGEN 1 mg/ml

For *in Vitro* **diagnostic use only**

Kit for evaluation of platelet aggregation on whole blood and on PRP, induced by Collagen

I. INTENDED USE

Collagen is for use in routine platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation.

II. PRINCIPLE

When collagen is added to platelet rich plasma or whole blood, it stimulates platelets to change shape and adhere to the collagen. The platelets release endogenous ADP and aggregate. After the addition of collagen there is a lag phase during which the collagen fibrils polymerize for platelet activation. The study of the aggregation on PRP is based on the evaluation of changes in transmittance in a plasma sample during the aggregation.

Aggregation study in whole blood is based on to the evaluation of the electric resistance changes. Two electrodes while immersed in the sample are rapidly covered with platelets which, at the very first contact, appear in shape of monolayer. When the aggregating agent is added, additional platelets tend to attach the monolayer previously formed, determining an impedance increase between the two electrodes.

III. REAGENTS AND MATERIALS

Each kit contain:

1. Collagen 1 mg/ml: suspension with 1 mg/ml of native collagen fibrils from equine tendon in isotonic glucose solution.
2. Diluent B: isotonic glucose solution, pH 2,7-2,9.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Blood collection tubes, centrifuge tubes, tubes and pipettes for drawing up the blood and the PRP, all in siliconized glass or plastic.
- Aggregometer.

IV. STORAGE

Store diluent and collagen tightly closed in refrigerator (2-8°C),. The kit is stable until expiration date printed on the package label.

V. SAMPLE COLLECTION

Collect the sample from an antecubital vein without stasis using siliconized needle 18 or 20 G. Immediately transfer blood into a plastic tube containing the anticoagulant (trisodium citrate 3.8%) in the ratio of 1/9 (v/v). The blood can be used as it is for platelet aggregation within 3 hours.

To obtain PRP centrifuge the blood at 160 g for 10 minutes, carefully draw off the supernatant (PRP); centrifuging time and speed depend on the kind of sample; inspect visually the supernatant. In case of red cells presence, centrifuge again. Collect the supernatant (PRP) with the plastic pipette and store in a plastic test tube, identified with the proper label, and carry out a platelet count. Re-centrifuge the remaining citrated blood at 2000 g for 30 minutes and decant the supernatant (PPP). Collect the supernatant (PPP) with the plastic pipette and store in a plastic test tube, identified with the proper label, until the analysis. Dilute PRP with PPP to obtain a plasma with about 300.000 platelet/mm^3. Maintain the PRP at room temperature and carry out the test within 3 hours.

VI. PRP TEST PROCEDURE

Mix by inverting the collagen 1 mg/ml and dilute 0.1 ml with 0.4 ml of Diluent B. Concentration of work solution: 200 µg/ml. This solution is stable few hours in a bath of melting ice.

1. Prepare PRP and PPP as described in section V.
2. Add 500 µl (250 µl) of PRP to an aggregation cuvette containing stirring bar and incubate at 37°C for 3 minutes.
3. Add 500 µl (250 µl) of PPP to an aggregation cuvette without stirrer.
4. Place PRP and PPP cuvettes in corresponding instrument sample wells and follow manufacturer's instruction for setting base lines.
5. Bring collagen to room temperature and swirl to mix.
6. Add 10 µl (5 µl) diluted collagen to PRP cuvette.
7. Record platelet aggregation response for a minimum of 5 minutes.

The figures in parentheses are half volumes that a lot of aggregometers can now handle; using the proper rubber adhesive spacers.

VII. WHOLE BLOOD PROCEDURE

1. Add 500 µl of saline solution and 500 µl of whole blood with anticoagulant in a 1 ml plastic cuvette containing stirring bar and incubate at 37°C for 5 minutes.
2. After connecting the electrode to the socket, put incubated at 37°C for 5 minutes.
3. Alfter incubation, place it in the vial containing the diluted blood. (Place the filaments to the back of aggregometer).
4. Place the cuvette into the reaction well and incubate 2 minutes, holding the door closed. Open the door and pipette the amount of collagen careful pipette on the bottom, rinse several times (Warning: avoid formation of air bubbles).
5. Record platelet aggregation.

   Add 10 µl of diluted collagen to 1 ml of diluted sample to have a concentration of 2 µg/m
   20 µl of diluted collagen to 1 ml of diluted sample to have a concentration of 4 µg/ml
   25 µl of diluted collagen to 1 ml of diluted sample to have a concentration of 5 µg/ml.

It is recommended to work at two different concentrations of collagen: 2 µg/ml and 4 µg/ml .

VIII. INTERPRETING THE RESULTS

Normal values	Collagen in **PRP** -	Concentration 2 µg/ml:	% aggregation 70 - 94%.
	Collagen in **whole blood** -	Concentration 2 µg/ml:	aggregation (ohm) 15 – 27.
	Collagen in **whole blood** -	Concentration 5 µg/ml:	aggregation (ohm) 15 – 31.

IX. PERFORMANCES

This product will perform as described prior to its expiration date when procedural and storage directions are followed.

Linearity, accuracy, precision.

Platelet aggregation induced by common aggregating reagents (ADP, Arachidonic Acid, Collagen and Adrenaline) is a nonlinear test system for some parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by many factors such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reactants or their concentration.

In platelet aggregation, accuracy is a relative parameter and is dependent on the test system.

The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges.

X. NOTE

To test at the same time optical test on PRP and the release of ATP with **bioluminescent** technique should work on a **lumi-aggregometer**. (Example 700-2). Refer to the Technical Manual and the instructions in User Manual of instrument.

XI. REFERENCES

Refer to the Technical Manual **3115xxx BE-0 07/11**.

CONTENT	**REF. 311501C**	**REF. 311502C**
Collagen 1 mg/ml	6 x 0,5 ml	2 x 0,5 ml
Diluent B	6 x 4,0 ml	2 x 4,0 ml
Instruction for use		1 item

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (LXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

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