ADRENALINE 5 mM

For *in Vitro* diagnostic use only

Test for the evaluation of platelet aggregation on PRP induced by Adrenaline.

I. INTENDED USE
Adrenaline is for use in routine platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation.

II. PRINCIPLE
When Adrenaine is added to platelet rich plasma, it stimulates platelets to change shape and to aggregate. The aggregation induced by Adrenaine is primary aggregation. The normal platelets release endogenous ADP from its granules. The release of endogenous ADP occurs in a second wave of aggregation.

III. REAGENTS AND MATERIALS
Each kit contain:
1. Lyophilized 5 mM Adrenaline. Reconstitute each vial with 0,5 ml of bidistilled water.
2. Diluent A: buffer for dilution with TRIS, pH 7,3.

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.
- Bidistilled water.
- Aggregometer.

IV. STORAGE
Store the kit at +2 - +8°C. The kit is stable until expiration date printed on the package label. After reconstitution and taking of the amount required for the test, the vials of **adrenaline** should be removed. The **Diluent A** can be used more times.

V. SAMPLE COLLECTION
Collect the sample from an antecubital vein without stasis by slowly drawing up the blood with the syringe and slowly expelling it (after having removed the needle), into the collection tube; avoid haemolysis. Carry out the venepuncture with a plastic syringe and mix 9 volumes of blood with 1 volume of trisodium citrate 3.8% in a plastic or siliconized tube. Centrifuge the blood at 200 g for 10 minutes, carefully draw off the supernatant (PRP) and carry out a platelet count on this. Re-centrifuge the remaining citrated blood at 2000 g for 30 minutes and decant the supernatant (PPP). 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 4 hours.

VI. PREPARATION OF WORKING SOLUTION
Before use dilute two a 0,1 ml aliquots of **Adrenaline** (previously reconstitute) with 4,9 ml and 0,9 ml of **Diluent A** to obtain two working solutions at concentrations of 0,1 mM and 0,5 mM respectively. Working solution is stable for 60 minutes at RT. Each vial contains sufficient reagent to carry out **100 aggregation test.**

VII. TEST PROCEDURE
For a routine examination, carryout the test at 2 Adrenaline concentrations in order to induce a biphasic aggregation (1,0 µM) and an irreversible monophasic aggregation (10 µM).
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 Adrenaline to room temperature and swirl to mix.
6. Add 5,0 µl (2,5 µl) Adrenaline 0,1 mM to PRP cuvette to obtain biphasic aggregation. Add 10 µl (5,0 µl) Adrenaline 0,5 mM to obtain monophasic irreversible aggregation.
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.

VIII. INTERPRETING THE RESULTS
Normal subjects

Adrenaline - Concentrations less than 0,2 µM: aggregation reversible.
Adrenaline - Concentrations between 0,2 and 5,0 µM: aggregation biphasic with secondary wave induced by endogenous aggregating agents.
Adrenaline - Concentrations above 5,0 µM: aggregation monophasic irreversible **(% aggregation 70-80%).**

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
- **Epinephrine is not recommended as a standard agonist for whole blood testing clinically**. As a fewer than 50% respond to this very weak agonist.
- 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 **M3115xxx BE-0 07/11**

CONTENT	**REF: 311501BL**
Adrenaline 5mM lyphilized	9 x 0,5 ml
Diluent A	1 vial x 50 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

M311501BL DE-2 11/14 CE IVD

Mascia Brunelli S.p.A. Viale Monza 272, 20128 Milano, Tel. 02-25209.1, Fax 02-2576428, mktg@masciabrunelli.it www.masciabrunelli.it