ADP 0,1 mM

For *in Vitro* diagnostic use only

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

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

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

III. REAGENTS AND MATERIALS
Each kit contain:
1. Lyophilized ADP.
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.
- Aggregometer.

IV. STORAGE
Store 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 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. PRP TEST PROCEDURE
Reconstitute one vial with 0,5 ml of Diluent A. The lyophilized reagent once reconstituted is stable for 1 month at -20°C. To avoid repeated thawing and freezing it is advised to subdivide the solution into aliquots of 0,1 ml and freeze. With the work scheme proposed here, the reagent is sufficient to carryout **70 aggregation test/vial** (generally about 35 patients/vial).

For a routine examination, carryout the test at 2 ADP concentrations in order to induce a biphasic aggregation (0,8 µM) and an irreversible monophasic aggregation (2 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 ADP to room temperature and swirl to mix.
6. Add 4,0 µl (2,0 µl) diluted ADP to PRP cuvette to obtain biphasic aggregation. Add 10 µl (5,0 µl) diluted ADP 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.

VII. INTERPRETING THE RESULTS

Normal subjects	ADP -	Concentrations less than 0,3 µM:	aggregation reversible.
	ADP -	Concentrations between 0,3 and 1,5 µM:	aggregation biphasic.
	ADP -	Concentrations above 1,5 µM:	aggregation monophasic irreversible.
	ADP -	Concentration 5 µM:	69 – 88 %
	ADP -	Concentration 10 µM:	71 – 88 %

VIII. 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.

IX. 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.

CONTENT	**REF: 311501A**
ADP 0,1 mM	8 x 0,5 ml
Diluent A	1 vial x 5 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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