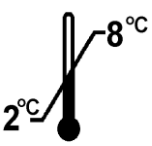
ADP Reagent

REF **HB-5501-FG (1x1ml)**
HB-5502-FG (2x1ml)

Guide to Symbols

Consult accompanying documents
Voir documents cijoints
Siehe beigefügte, Dokumente
Véanse los documentos djuntos
Vedi documenti allegati

For *in vitro* diagnostic use
Pour usage diagnostique *in-vitro*
in-vitro diagnostikum
Para uso diagnóstico *in-vitro*
Per uso diagnostico *in-vitro*



Store at 2-8°C
Conserver à 2-8°C
Lagern bei 2-8°C
Conservar a 2-8°C
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Use By
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(HB-0173-LIT Rev 4 July 11)

ADP Reagent

INTENDED PURPOSE GB Handling Instructions

Hart Biologicals ADP is used to diagnose platelet dysfunction, or normal platelet activity in human platelet rich plasma or whole blood.

SUMMARY

An important mediator of platelet activation and aggregation is ADP (adenosine 5'-diphosphate) which is released from blood platelets in the vasculature upon activation by various agents, such as collagen and thrombin, and from damaged blood cells, endothelium or tissues. Activation by ADP results in the recruitment of more platelets and stabilization of existing platelet aggregates. Platelet ADP receptors mediating aggregation are activated by ADP and some of its derivatives and antagonized by ATP (adenosine 5'-triphosphate) and some of its derivatives[1]. Therefore, platelet ADP receptors are members of the family of P2 receptors activated by purine and/or pyrimidine nucleotides[2].

TEST PRINCIPLE

The platelet aggregation test measures the rate and degree to which dispersed platelets in a sample of platelet rich plasma (PRP) or anticoagulated whole blood forms clumps (aggregates) after the addition of a substance that normally stimulates platelet aggregation (agonist). In optical aggregometry, the clumping of the platelets causes the platelet rich plasma to become less turbid . This is measured on a platelet aggregometer, which plots the rate and maximum extent of the aggregation reaction. In whole blood aggregometry, platelets adhere to small wires suspended in the blood sample and the impedance between the wires as the platelets adhere and aggregate is measured and plotted.

WARNINGS AND PRECAUTIONS

For *in-vitro* diagnostic use only.
Do not pipette by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
Wear disposable gloves when handling specimens and kit reagents, and wash hands thoroughly afterwards.

MATERIALS PROVIDED

ADP Reagent

Ingredients: The reagent contains a lyophilised preparation of 200µM ADP with added buffer and stabilisers.
Preparation for use: Reconstitute each vial of ADP reagent with 1.0ml of distilled or deionised water as indicated on the vial label. Allow to stand for 10 minutes and mix well before use.
Storage and stability: The lyophilised product should be stored at 2…8°C and is stable until the expiry date printed on the vial label. After reconstitution, the product is stable for 8 hours at room temperature (20…25°C) , 2 weeks at 2..8°C or 4 weeks at -20°C.

MATERIALS REQUIRED BUT NOT PROVIDED

Platelet aggregometry system – the Hart Biologicals ADP Reagent will perform satisfactorily when used on any aggregometer system. Follow the manufacturer's instructions for the operation of the aggregometer in use.
Purified water.
Pipette for 1.0ml

SAMPLE COLLECTION AND PREPARATION[4]

Preparation of Platelet-Rich and Platelet- Poor Plasma for Optical Aggregation

Blood for platelet aggregation testing should be collected in to plastic syringes and transferred to plastic tubes, or collected in siliconised glass evacuated blood collection tubes.
Blood (9 parts) should be mixed with 0.11M or 0.13M sodium citrate anticoagulant (1 part). Invert gently to mix. Do not shake.

- Prepare platelet rich plasma by centrifuging the anticoagulated blood at 150-200 x g for 10-15 minutes at room temperature.
- Remove the platelet rich plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PRP'. Cap the container and keep at room temperature.
- Prepare platelet poor plasma by centrifuging the remaining blood specimen at 2000 x g for 20 minutes.
- Remove the platelet poor plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PPP'. Cap the container and keep at room temperature.
- Adjust the platelet concentration in the PRP to 200-300x10^9/L using PPP, cap and allow to stand at room temperature for 30 minutes prior to testing.
- Testing should be completed within 3 hours of blood collection.

Whole Blood Aggregation Samples

Refer to the aggregometer manufacturer recommendations for the preparation of samples for whole blood aggregometry.

TEST PROCEDURE

A) Optical Aggregometry

1. Set the 0% and 100% aggregation levels on the aggregometer using platelet poor plasma and platelet rich plasma according to the manufacturers instructions.
2. Pipette the required volume of platelet rich plasma in to an aggregation cuvette and add a stir bar.
3. Pre-warm to 37°C for 120 seconds.
4. Add the required volume of ADP directly in to the cuvette. Do not allow reagent to run down the wall of the cuvette.
5. Allow the aggregation pattern to form for a minimum of 5 minutes.

B) Whole Blood Aggregometry

Refer to the manufacturers instructions for the correct performance of the test.

QUALITY CONTROL

The results of platelet aggregation studies should be interpreted against the results of aggregation profiles of a normal sample tested at the same time. The normal donor should not have ingested aspirin or aspirin containing compounds in the preceding 10 days and should not be on any other form of anti-platelet medication.

EXPECTED VALUES [5,6]

Low concentrations of ADP (<0.5 to 2.5µM) cause primary or reversible aggregation. ADP binds to a platelet membrane receptor and releases calcium ions. A reversible complex with extracellular fibrinogen then forms which causes a shape change and then a reversible aggregation. At very low concentration s of ADP, platelets may disaggregate after the initial primary phase. With higher concentrations of ADP, an irreversible second wave of aggregation, associated with the activation of the arachidonic acid pathway and the subsequent release of dense and alpha granule contents. The expected response to ADP in the most commonly encountered defects are listed below:

Condition	**ADP Aggregation**
Thrombasthenia	Absent
Bernard-Soulier syndrome	Normal
Storage Pool defect (δ)	Primary wave only
Cyclo-oxygenase deficiency	Normal or Primary wave only
Thromboxane synthetase deficiency	Normal or Primary wave only
Aspirin ingestion	Primary wave only
Ehlers-Danlos syndrome	Normal
Von Willebrand disease	Normal

If only high doses of ADP are used, defects in the primary wave of aggregation could be missed.

FURTHER TESTING

If the test results are abnormal, the test should be repeated on a separate occasion. If the results are consistently abnormal, and the patient is not taking any medication known to interfere with platelet function, additional tests should be considered [6].

LIMITATIONS

In optical aggregometry, the presence of red blood cells in the PRP will cause the total observed aggregation to be reduced. The presence of platelets in the PPP will cause the total observed aggregation to appear increased.
Spurious results can be observed when the total platelet count of the PRP is less than 75 x 10^9/L.
PRP tested less than 30 minutes after preparation may exhibit abnormal aggregation profiles.

Bibliography

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3. Dacie & Lewis, 'Practical Haematology', Lewis, S.M., Bain, B.J. and Bates, I. (Editors); 9th Edition, Elsevier Science Ltd., 2002, pages 380-381.
4. Day, H.J. and Holmsen, H., 'Laboratory Tests of Platelet Function', Ann. Clin. Lab. Sci., 1972; 2 : 63.
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6. Dacie & Lewis, 'Practical Haematology', Lewis, S.M., Bain, B.J. and Bates, I. (Editors); 9th Edition, Elsevier Science Ltd., 2002, pages 384-385.