For in vitro research use only

multiplate®

ADPtest

for platelet aggregation studies

ADP reagent kit

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Valid for REF MP0120, MP0220, MP0192

V.4.0-US-RUO Revised 2012-02

Product description

The ADPtest reagent is a lyophilized preparation of adenosine-5'-diphosphate, stock concentration 0.2 mM.

Intended use

The ADPtest reagent is for use in routine platelet aggregation studies for the evaluation of normal platelet function.

Principle

When added to a platelet sample, ADP triggers platelet activation via a platelet's ADP receptors. Exposure to exogenous ADP will cause normal platelets to release endogenous ADP from their granules and result in irreversible aggregation.

Materials provided

The reagent is provided in one of three kit formats:

MP0120: ADPtest reagent, 1 x 1.0 ml,
with 5 color coded micro test tubes for aliquotation.

MP0192: ADPtest reagent, 1 x 1.0 ml.

MP0220: ADPtest reagent, 3 x 1.0 ml.

Materials required but not provided

1. Platelet aggregometer
2. Purified water (distilled or deionized)
3. Aggregometer test cells with stir bars
4. Micropipettes – 0.5 µl to 100 µl required for reagents
5. Pipettes – 100 µl to 1 ml required for blood samples
6. Physiological saline (NaCl 0.9%) for irrigation/$CaCl_2$ (MP0530) for the dilution of whole blood sample

Instrumentation

ADP will perform as described when used on the Multiplate® Aggregometer. Follow the manufacturer's instructions.

Reagent preparation

Reconstitute each vial of ADP with 1.0 ml of high purity (distilled or deionized) water. Allow to stand at room temperature for 10 minutes and swirl gently to mix – do not shake! The solution should be clear and colorless.

Note: Due to risk minimization procedures the vacuum in the vials was replaced by an inert gas.

Storage and stability

Lyophilized Reagent: until the expiry date printed on the vial label when stored at 2-8°C.

Reconstituted Reagent: for 7 days when stored at 2-8°C. When stored at < -20°C the reconstituted reagent is stable for 4 weeks. Reconstituted vials should remain tightly closed when not in use. It is advisable to minimize exposure to light, air and elevated temperatures.

For optimal handling, reconstituted reagent may be aliquoted and the aliquots stored frozen at < -20°C.

Freshly thawed aliquots are stable for 24 hours at room temperature after one freeze thaw cycle.

Warnings and precautions

The ADPtest reagent is for In-vitro-research use only and not for injection or ingestion. Observe standard precautions when handling test specimens and all test materials. Dispose of all waste materials according to local regulations.

Sample collection

Blood collection should be performed with caution to avoid prolonged venous stasis and using a large-bore needle during draw. Collect samples into sterile evacuated tubes with non-wettable lining containing 1/10 volume of 3.2% buffered sodium citrate. Avoid foam formation in the blood collection tube. Always ensure citrated blood collection tubes are filled to the indicated fill volume, in order to avoid excessive citrate levels. Gently invert the collection tube 4 to 5 times to ensure complete mixing of the contents. Do not freeze or refrigerate samples. Do not preheat the blood before analysis.

Test procedure

Final working concentration: 6.5 µM ADP

1. Insert a disposable test cell with stir bar
2. Dilute 300 µl of whole blood (room temperature) with 300 µl NaCl/$CaCl_2$, preheated at 37°C
3. Incubate diluted blood for 180 sec.
4. Add 20 µl of stock ADPtest reagent.
5. Start test
6. Measure test for 6 min

Note: It is important that the tip of the micropipette is immersed in the sample when the reagent is injected. The saline (NaCl 0.9%) must not contain any additives such as methyl ester. This can cause false-positive results. It is important to pay close attention to temperatures and incubation times. The use of non-preheated NaCl/$CaCl_2$ diluent solution or the introduction of shorter incubation times may skew results.

When using the Multiplate® electronic pipette in auto mode follow the test instructions displayed by the Multiplate® software.

Quality Control

Laboratories should follow generally accepted quality control practices when proficiency testing is not available. It is good laboratory practice to run a drug-free normal control whenever reagents are reconstituted or thawed.

Expected results

Clopidogrel inhibits ADP-induced aggregation. Paniccia et al. investigated 297 patients taking clopidogrel 75 mg / d and reported ADP induced aggregations (10 µM) of 26.8 ± 15.8 U (mean ± SD). The median was 22 U and the range 0-91 U.

Glanzman thrombasthenia leads to reduced ADP induced aggregation. 5 patients with Glanzman thrombasthenia were tested and the following aggregations were determined (median and range): 5 U (1-6 U).

Interpretation of results

Aggregation curves in blood can be interpreted as follows:

- By direct comparison to a normal drug free control that also provides real time quality control
- By comparison to published normal values that can be verified and reproduced by any laboratory

Normal ranges

Note: The following normal range was obtained by analyzing 260 individuals in three centers. It should be used as a guideline only. Normal ranges should be established in each laboratory.

Normal range in whole blood (90% Confidence Interval)		
Reagent	**Concentration**	**AUC (U)**
ADP	10 µM	43-92

Limitations

Samples should be analyzed within the period of 0.5-3 hours after blood collection.

Many drugs inhibit platelet function. Unless the aim of testing is to demonstrate drug-induced inhibition, patients should be drug free for two weeks prior to testing.

A detailed patient history is required for accurate test interpretation. Patients should be questioned about recent ingestion of any medication.

The platelet count in the test sample must be above 100,000 when testing in whole blood.

Literature

Tóth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. Thromb Haemost. 2006 Dec; 96(6): 781-8.

Paniccia R, Antonucci E, Maggini N, Romano E, Gori AM, Marcucci R, Prisco D, Abbate R. Assessment of platelet function on whole blood by multiple electrode aggregometry in high-risk patients with coronary artery disease receiving antiplatelet therapy. Am J Clin Pathol. 2009 Jun; 131(6): 834-42.

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