ADP

REF AG001K-RUO

R 3 x 0.1 µmol

ADP for platelet aggregation tests
FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 06-2021

INTENDED USE:

Measurement of platelet aggregation.
This kit should be used for research use only and must not be used for patient diagnosis or treatment.

PRINCIPLE:

When added to platelet-rich plasma (PRP), adenosine 5'-diphosphate (ADP) binds to $P2Y_1$ and $P2Y_{12}$ receptors at the platelet surface, inducing a two-phase aggregation process. ADP-mediated platelet activation leads to a change in platelet shape and induces an initial wave of aggregation. If the ADP concentration is sufficiently high, a second wave of aggregation occurs, following the release of the contents of platelet granules, consisting amongst others of serotonin, fibrinogen, thromboxane and endogenous ADP. ADP is considered to be a weak agonist as the effects it produces are partial and reversible.

REAGENTS:

R **Adenosine-5'-diphosphate (ADP):** Lyophilized ADP, contains Tris and stabilizing agents. **3 x 0.1 µmol vials.**

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30 °C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- To ensure optimum test results, we recommend testing the specimens and controls in succession and without interruption.
- For *in vitro* use.

REAGENT PREPARATION AND STABILITY:

The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial, gently remove the freeze-drying stopper.

R **ADP**
For use with the aggregometer:
Reconstitute the contents of each vial with **exactly 0.5 mL distilled water**, shake vigorously until fully dissolved. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.

For use with the analyzer:
Reconstitute the contents of each vial with **exactly 0.625 mL distilled water**, shake vigorously until fully dissolved. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C), shaking occasionally.
Homogenize the reagent prior to use.

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- **4 weeks** at 2-8 °C.
- **24 hours** at room temperature (18-25 °C).
- **2 months** frozen at -20 °C or less*

*Thaw only once, as rapidly as possible at 37 °C, adapting the incubation period to the volume of reagent. The stability of the thawed reagent should be checked under laboratory work conditions.

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8 °C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.
- Saline solution (0.9% NaCl).

Materials:
- Light transmission Aggregometer.
- Sysmex CS-series analyzer and associated consumables.
- Calibrated pipettes.

SPECIMEN COLLECTION AND PREPARATION :

Specimens should be prepared and stored in accordance with applicable local guidelines.
- Specimens:
Human plasma obtained from anticoagulated blood (trisodium citrate).

- Collection:
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube. Cover and gently invert 4 to 5 times to mix. **Keep the specimens at room temperature** (~18 to 25 °C). Do not use CTAD tubes for collection.
- Centrifugation:
Preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP):
1) Prepare the PRP by centrifuging the blood specimens onto citrate at 150 x g for 10 minutes at room temperature (18-25 °C).
2) Examine the plasma: if any red blood cells are left, re-centrifuge at 150 x g for 5 additional minutes.
3) Using a plastic pipette, identify and carefully remove the platelet layer without touching the PRBC (leukocytes and red blood cells) and transfer to an identified tube (PRP). **Seal and store at room temperature**.
4) Prepare the PPP by centrifuging the remaining blood specimen at 2000-2500 x g for 15 minutes. Examine the PPP for haemolysis, then transfer to a plastic tube identified 'PPP'. **Seal and store at room temperature**.
5) See ISTH guidelines for the PRP platelet count.

PROCEDURE:

Automated method:
The application for Sysmex CS-series analyzers is available on request. **See the application and precautions specific to each analyzer.**

Aggregometer: ADP dilution:
To evaluate platelet aggregation in response to ADP, this latter is tested at concentrations ranging from 1 to 10 µM (final concentration, in the test). Prepare a sufficient quantity of the following 10X concentrated dilutions and test them as per the protocol below, starting with the highest concentrations:

"10X" ADP preparation (µM)	100	50	20	10
ADP 200µM (µL)	200	100	40	40
Saline solution (µL)	200	300	360	760
Giving a final concentration in the test (µM)	**10**	**5**	**2**	**1**

Protocol:
The test must be performed within 3 hours of specimen collection.

1. Place a stirrer in each cuvette.
2. Establish the 100% aggregation point with a cuvette containing **360 µL PPP**.
3. Pipette **360 µL** platelet-rich plasma (PRP) into a second cuvette. Incubate for **2 minutes at 37 °C**. Establish the 0% aggregation point with the **PRP**.
4. Add **40 µL ADP (10X)** directly into the platelet-rich plasma using a long and fine pipette tip. **Do not inject against the walls of the cuvette**.
5. Allow the aggregation profile to develop for 5 to 10 minutes.

Each laboratory can establish and validate its own test protocol and verify the resulting performance under its own specific working conditions (reagents/instruments/test protocol combination).
The user is responsible for validating any changes and their impact on all results.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
The control should be prepared in the same manner as the specimens. For qualitative platelet aggregation studies, the control may consist of fresh platelet-rich plasma collected from a normal (specified and qualified) donor who has not taken any aspirin or equivalent for the past 10 days and with a history of normal platelet function.
Include the quality controls with each test, as per good laboratory practice, in order to validate the test. A new control should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptable range for the method.
Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
The results obtained should be used for research use only and must not be used for patient diagnosis or treatment.

PERFORMANCE:

Example of maximum, normal and abnormal aggregation (%):

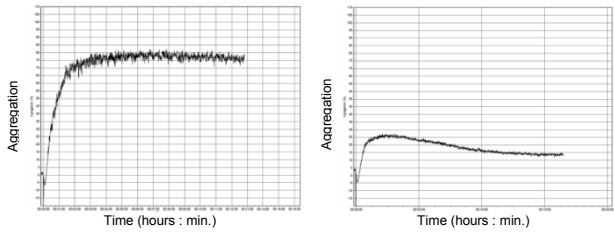|	Normal	Abnormal
Maximum aggregation (%)	80	20

Figure: Example of normal (left) and abnormal (right) aggregation plots with ADP (5 µM).

REFERENCES:

1. Zhou *et al.*, "Platelet aggregation testing in platelet-rich plasma". AM J Clin Pathol, 123:172-183, 2005.

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.