Epinephrine

REF AG002K-RUO

R 3 x 0.5 µmol

HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

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

English, last revision: 02-2025

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), epinephrine binds to the α_2-adrinergic receptors on the platelet surface, inducing an initial wave of reversible aggregation. This primary aggregation leads to exposure of fibrinogen receptors and inhibition of adenylate cyclase activity. A second wave of aggregation, clearly distinct from the first, is triggered by ADP release from δ granules and by thromboxane A_2 synthesis. Epinephrine is considered to be a weak agonist as the effects it produces are partial and reversible[1].

REAGENTS:

R **Epinephrine**: lyophilized, contains Tris, epinephrine hydrogen tartrate and stabilizing agents.
3 x 0.5 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.
- To ensure optimum test results, we recommend testing the specimens and controls in succession and without interruption.
- Toxic product. This product must be handle with all necessary precautions, wearing appropriate laboratory clothing, safety glasses and gloves. Product contact with skin and ingestion must be avoided.
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.
- P264 : Wash thoroughly after handling.
- P301 + P310 : IF SWALLOWED: Immediately call a POISON CENTER/ doctor.
- P330 : Rinse mouth.
- For *in vitro* use.

Danger! H300 : Fatal if swallowed

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 **Epinephrine**
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:

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

"10X" Epinephrine preparation (µM)	100	50	20	10
Epinephrine (µL) (**conc.**)	100 (**1 mM**)	50 (**1 mM**)	200 (**100 µM**)	100 (**100 µM**)
Saline solution (µL)	900	950	800	900
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 epinephrine (10X)** directly into the platelet-rich plasma using a long and fine pipette tip. **Do not inject the epinephrine 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:

Commercial controls are not available.
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 series, 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.
- If the number of platelets is lower than 150 × 109/L or higher than 600 × 109/L, test results may be affected. The platelet count of PRP samples should not be adjusted to a standardized value with autologous PPP[2].

The results obtained should be used for research use only and must not be used for patient diagnosis or treatment.

RESULTS:

- Results are evaluated by examining the aggregation curve and the maximal aggregation (%). These parameters vary depending on instrument type, and specific normal values should be determined by each laboratory.
- Abnormal curves should be confirmed via a retest.
- Lot to lot variability measured on 3 lots is %CV ≤ 10% (normal sample).

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

	Normal	Abnormal
Maximum aggregation (%)	92	32

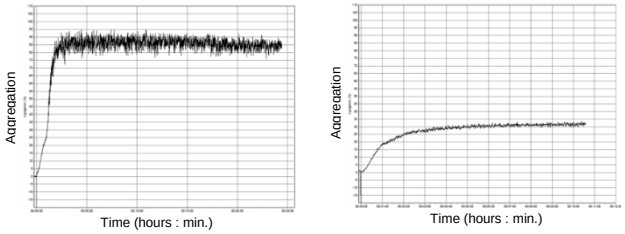

Figure: Example of normal (left) and abnormal (right) aggregation plots with Epinephrine (10 µM).

REFERENCES:

1. Zhou *et al*., "Platelet aggregation testing in platelet-rich plasma". AM J Clin Pathol, 123:172-183, 2005.
2. Cattaneo M. *et al.* Recommendations for the Standardization of Light Transmission Aggregometry: A Consensus of the Working Party from the Platelet Physiology Subcommittee of SSC/ISTH. J Thromb Haemost. 2013.

SYMBOLS:

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

Changes compared to the previous version