Lyophilized Platelets

REF AG006K

R1 R2 3 x 5 mL

Platelets for Ristocetin cofactor activity assay (vWF:RCo)

Not for Sale in the US

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INTENDED USE:

For *in vitro* diagnostic use. Measurement of platelet aggregation.

SUMMARY AND EXPLANATION:

The Lyophilized Platelets reagent can also be used with Ristocetin (AG004K) for the Ristocetin co-factor activity assay (vWF:RCo), to assist in the diagnosis of von Willebrand disease.

PRINCIPLE:

When added to a suspension of platelets fixed in a platelet-poor plasma, Ristocetin promotes interaction between von Willebrand factor (vWF) and its platelet receptor, glycoprotein GPIb-V-IX. The vWF:RCo test measures the biological activity of vWF by agglutination of fixed platelets at a given Ristocetin concentration. Platelet agglutination is thus dependent upon the concentration of vWF in the plasma.

REAGENTS:

R1 **Reagent 1: Formaldehyde-fixed platelets**: lyophilized in the presence of stabilizing agents.
3 x 5 mL vials.
R2 **Reagent 2: Tris-NaCl buffer (Tris-buffered saline - TBS)**: for reconstituting the lyophilized platelets (contains BND as a stabilizing agent).
3 x 5 mL 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.
- The usual laboratory health and safety procedures must be followed.
- For *in vitro* diagnostic 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.

R1 **Reagent 1: Platelets**

Reconstitute the contents of each vial with **exactly 5 mL of** R2 **Tris-NaCl buffer (TBS) (0.05 M Tris, 0.15 M NaCl, pH 7.35)** and shake vigorously until completely 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:

- **56 days** at 2-8 °C.
- **7 hours** at room temperature (18-25 °C).
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R2 **Reagent 2: Tris-NaCl buffer (Tris-buffered saline - TBS)**

Ready to use. Allow the reagent to stabilize for 30 min. at room temperature (18-25 °C) before use.
Homogenize thoroughly before use.

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:

- Saline solution (0.9% NaCl).

Materials:

- SD Medical aggregometer (or equivalent), used as per manufacturer's guidelines, with appropriate stirrers and aggregometry cuvettes.
- Sysmex CS-series analyzer and associated consumables.
- Calibrated pipettes.

PROCEDURE:

The reconstituted platelets can be used as a source of platelets for most Ristocetin cofactor activity test procedures.
The following protocol is given as an example only and must be validated for the laboratory's specific working conditions (reagents/instruments/test protocol combination).

1.	Place a magnetic stirrer in each cuvette
2.	Prepare a blank by pipetting **150 µL** platelet-poor plasma (**PPP**) + **150 µL** saline solution into a cuvette. Establish the 100% aggregation point with the blank.
3.	Pipette **270 µL** of platelets into a second cuvette.
4.	Add **30 µL** of a **12 mg/ml** ristocetin solution. Incubate for **approximately 3 minutes at 37 °C**. Establish the 0% aggregation point with the platelets + Ristocetin mixture.
5.	Add **30 µL of the patient's plasma, or of calibrator plasma, diluted 1:2, 1:4, 1:8 and 1:16 in saline solution,** directly to the mixture. **Avoid pipetting the specimen down the walls of the cuvette.**
6.	Record aggregation profile for at least 6 minutes.

The user is responsible for validating any changes and their impact on all results.

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.

EXPECTED VALUES:

The vWF assay (activity and antigen) is used in the diagnosis of von Willebrand disease (vWD). There are three main types of von Willebrand disease, with varying severity:

- Type 1 (50 to 75% of cases), the mildest, is due to a partial quantitative vWF deficiency.
- Type 2 (20 to 30% of cases), generally more severe, is due to a qualitative vWF deficiency. There are 4 variants of type 2 vWD: 2A, 2B, 2M and 2N
- Type 3 (less than 5% of cases), the most severe, is due to the quasi-total absence of vWF and is associated with a profound FVIII deficiency[2-3].

PERFORMANCE:

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

	Normal	Abnormal
Maximum aggregation (%)	76	13

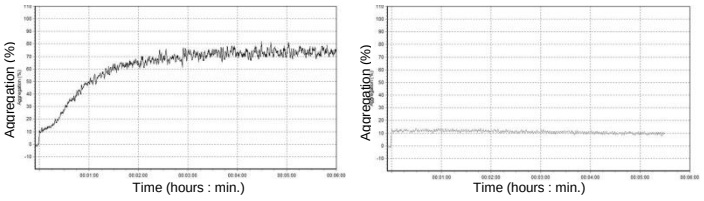

Figure: Example of normal (left) and abnormal (right) aggregation plots with Ristocetin (1.2 mg/mL).

REFERENCES:

1. Thompson, J.M. Blood coagulation and haemostasis, a practical guide, third edition, Longman group (FE) pg 142, 145, 192, 1985.
2. The Diagnosis, Evaluation, and Management of von Willebrand Disease. NIH Publication No. 08-5832, 2007.
3. Blatt, P.M. *et al*., Antihaemophilic factor concentrate therapy in vWD, J Am Med Assn, 236:2770-2772, 1976.

SYMBOLS:

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