

ADIAflo™ Platelet GpIb/IX/V

Kit for platelet membrane glycoprotein quantitation using flow cytometry.
Limited to Research Use Only in the USA

Product No. 672

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INTENDED USE

For single color flow cytometric analysis of platelet glycoproteins GpIb α , GpIX, and GpV. The number of glycoprotein antigenic sites is determined by converting the fluorescence intensity into the corresponding number of sites per platelet based on a calibrated bead standard curve.

REAGENTS

- Reagent 1:** 1 vial, 15 mL, diluent, 10X concentrated.
- Reagent 2a:** 1 vial, 100 μ L, negative isotypic control (mouse monoclonal antibody IgG).
- Reagent 2b:** 1 vial, 100 μ L, anti GpIb α MAb (CD42b).
- Reagent 2c:** 1 vial, 100 μ L, anti GpIX MAb (CD42a).
- Reagent 2d:** 1 vial, 100 μ L, anti GpV MAb (CD42d).
- Reagent 3:** 1 vial, 200 μ L, calibrated bead suspension. The beads are coated with known quantities of mouse IgG. The number of determinants coated on each bead population is indicated on the box label of the kit. These values may vary from lot to lot.
- Reagent 4:** 1 vial, 500 μ L, staining reagent, polyclonal anti-mouse IgG-FITC.

WARNING: All reagents contain sodium azide as a preservative and should be discarded with care to prevent the formation of explosive metallic azides. When dumping waste materials into a sink, use copious quantities of water to flush plumbing thoroughly.

REAGENT PREPARATION AND STORAGE

Unopened vials remain stable until the expiration date printed on the box label, when stored at 2-8°C (**DO NOT FREEZE**).

Reagent 1

- Stability after opening: 2 months at 2-8°C when free of contamination.
- The presence of crystals does not affect the quality of the reagent. Incubate at 37°C until the crystals are completely dissolved.
- Prepare working strength reagent by making a 1:10 dilution with deionized water. Prepare sufficient volume for the test series.
- Stability after dilution: 15 days at 2-8°C.

Reagents 2a, 2b, 2c, 2d and 4

- Ready for use.
- Stability after opening: 2 months at 2-8°C when free of contamination.

Reagent 3

- Ready for use after resuspension by vortexing for **5 seconds**.
- Stability after opening: 2 months at 2-8°C when free of contamination.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Collection

- Use polypropylene or siliconized glass blood collection tubes.
- To maintain platelet integrity, avoid activation by exercising utmost care during the collection procedure.
- The blood is to be collected in anticoagulant: 0.109 M or 0.129 M trisodium citrate (using a 9:1 volume ratio).

Preparation

- The test is performed on citrated whole blood or on platelet rich plasma (PRP) tested 1:2 with Reagent 1.

Storage

- The blood sample must be tested within **3 hours** after collection in order to avoid platelet activation.
- The sample should remain at room temperature before testing (18-25°C).
- Do not freeze the sample.

PROCEDURE

Exercise care in the pipetting of small reagent volumes by depositing them at the bottom of the test tubes. All reagents should be at room temperature before use.

1. Reagent tube setup

- a) Label 6 plastic tubes T1 to T6. Set the tubes in a rack.
- b) Perform the following pipetting steps:
 - i. Into tube T1: pipette **50 μ L** of the sample and add **150 μ L** of Reagent 1. Homogenize using a vortex.
 - ii. Into tube T2: pipette **20 μ L** of Reagent 2a (Negative control),
 - iii. Into tube T3: pipette **20 μ L** of Reagent 2b (GpIb α MAb),
 - iv. Into tube T4: pipette **20 μ L** of Reagent 2c (GpIX MAb),
 - v. Into tube T5: pipette **20 μ L** of Reagent 2d (GpV MAb),
 - vi. Into tube T6: pipette **40 μ L** of Reagent 3 (vortex vial well before pipetting).

2. Immuno-labelling of Samples and Control

- a) To each of tubes T2, T3, T4 and T5:
 - i. Add **20 μ L** of diluted sample from Tube T1.
 - ii. Homogenize the tubes using a vortex.
- b) Incubate at room temperature for **10 minutes**.

3. Fluorescent Staining

- a) To each of tubes T2 to T6:
 - i. Add **20 μ L** of Reagent 4.
 - ii. Homogenize using a vortex.
 - iii. Incubate at room temperature for **10 minutes**.
- b) Add **2 mL** of Reagent 1 into each of tubes T2 to T6.
- c) Prepared samples may be stored for 2 hours at 2-8°C before analysis.

INTERPRETATION AND REPORTING OF RESULTS

Cytometric analysis:

- Refer to the Operator's Manual of the cytometer for instructions on how to perform cytometric readings.
- The selected Mean Fluorescent Intensity (MFI) statistic is the geometric mean, called Mn(x) or GeoMean depending upon the cytometer used.
- Vortex each tube before analysis.

Calibration analysis: Tube T6 (Fig 1)

Create an FS LOG vs. SS LOG cytogram. Add a discriminator to minimize the artifact background. Set up a gate ("A") around the main single bead population (Fig 1a). Create an FL1 LOG histogram gated by the "A" region.

Note that the MFI for each of the 4 fluorescence peaks (Fig 1b: curves B, C, D and E) corresponds to the 4 calibration beads. For optimum analysis conditions, the peak of the fourth bead fluorescence intensity (FL1) must be set at the beginning of the fourth decade. To achieve this adjust the FL1 PMT voltage.

Fig 1a: Calibration cytogram

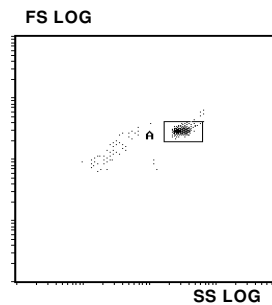
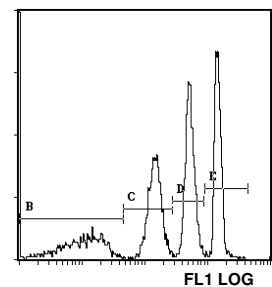


Fig.1b: Cursor settings in gated fluorescence histogram



(...over)

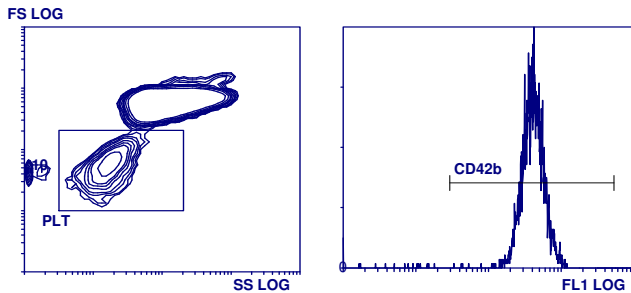
Sample analysis: Tubes T2 to T5 (Fig 2)

Using the same acquisition procedure, on the FS LOG vs. SS LOG cytogram (Fig 2a) platelets are isolated from other whole blood cells by an analysis region "PLT".

In the corresponding gated FL1 LOG fluorescence histogram (Fig 2b), note the mean fluorescence intensity of the positive peak for each assay, corresponding to 90% positive cells.

Fig 2a: Whole blood cytogram and platelet region gating

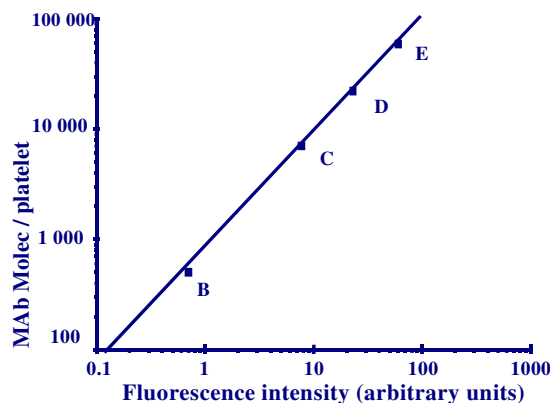
Fig 2b: CD 42b immuno-labelling, Cursor settings in PLT gated histogram



RESULTS

If the MFI are expressed as linear values, use a log-log plot. If the MFI values are obtained as channel numbers, use a semi-log plot. Plot the MFI calibration values (tube T6) on the abscissa (x-axis) and their corresponding number of monoclonal antibody molecules (as indicated on the box label) on the ordinate (y-axis). Draw the calibration curve.

Example Calibration Curve



Interpolate the MFI values of the tubes T2 to T5 on the calibration curve and read the corresponding numbers of monoclonal antibodies directly off the curve. Specific GpIb α , GpIX and GpV values are calculated after subtraction of the negative control measurement.

EXPECTED VALUES

GLYCOPROTEIN	NUMBER OF SITES
GpIb α	38,000 +/- 10,000
GpIX	30,000 +/- 7,500
GpV	15,000 +/- 6,200

Values are expressed as numbers of platelet bound molecules x 10³

LIMITATIONS

The presence of heparin in the sample may induce an under expression of glycoproteins GpIb α , GpIX, GpV.

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The following abstract was presented at the meeting of the International Society of Thrombosis and Haemostasis, August 1999:

Ledford, M., *et al.* Glycoproteins Ib, IX, V and CD62P are not activated or inhibited by an oral GpIIb/IIIa anti-platelet agent given to patients with vascular disease. Abst. 2602.