PLATELET Gp Screen

Kit for platelet glycoprotein quantitation

Kit for 10 determinations **Ref. 7008**

BioCytex

For Research Use Only.
Not for Use in Diagnostic Procedures.

1 INTENDED USE

Single color flow cytometric analysis of the platelet glycoproteins GpIIIa, GpIb and GpIa. The number of antigenic sites are determined by converting the fluorescence intensity into corresponding number of sites per platelet based on a calibrated bead standard curve.

2 EXPECTED VALUES

The cytometric analysis was performed on Coulter EPICS XL-MCL cytometer. These values are given as an indication only.
It is recommended that each laboratory establishes its own normal values from a local population of normal donors.

	Citrate
GpIIIa (CD61)	53 +/- 12
GpIb (CD42b)	38 +/- 11
GpIa (CD49b)	5 +/- 2.8

These values are expressed as number of Gp molecules per platelet (x 10^3)

3 REAGENTS

- **Reagent 1:** 1 x 15 mL vial, diluent, 10 fold concentrated.
- **Reagent 2a:** 1 x 200 µL vial, anti GpIIIa MAb (CD61).
- **Reagent 2b:** 1 x 200 µL vial, anti GpIb MAb (CD42b).
- **Reagent 2c:** 1 x 200 µL vial, anti GpIa MAb (CD49b).
- **Reagent 3:** 1 x 400 µL vial, calibrated bead suspension, ready for use. The beads are coated with increasing and accurately known quantities of mouse immunoglobulins IgG. The number of determinants coated on each bead population is indicated in the calibration flyer inserted in the kit.
- **Reagent 4:** 1 x 800 µL vial, staining reagent, polyclonal antibody anti mouse IgG-FITC.

4 REAGENT PREPARATION AND STORAGE

Intact kits and contents remain stable until the expiration date printed on the box label, when stored at 2-8 °C.*

- **Reagent 1 ** :** Stability after opening: 2 months at 2-8 °C when free of contamination.
  Prepare a **1:10 dilution** with distilled water. Prepare the appropriate volume required for the series to be tested.
  Stability after dilution: 15 days at 2-8 °C.
- **Reagents 2a, 2b, 2c 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.

Notes: * : do not freeze the kit.
** the presence of crystals does not affect the quality of the reagent. Incubate at 37 °C until the crystals are completely dissolved.

5 SPECIMEN COLLECTION AND TREATMENT

- **Sample collection:**

- Use non-wettable plastic blood collection tubes.
- In order to maintain platelet integrity, exercise utmost care to avoid platelet activation during the collection procedure.
- Anticoagulant : trisodium citrate 0.109 M or 0.129 M (using a ratio 9:1 volumes).

- **Sample storage:**

- Blood sample must be treated within **8 hours** after collection. Blood is stored at room temperature before testing (18-25 °C).
- The test is performed either on citrated whole blood or on platelet rich plasma (PRP).

6 PROCEDURE

Note: For good results exercise great care in the pipetting of small reagent volumes (20 µL) by depositing them at the bottom of the test tubes.
All reagents must be at room temperature.

6.1. Reagent Tubes Setup

Take 5 plastic tubes; mark them T1 to T5. Set the tubes in a rack.
Perform the following pipetting steps:

- Tube T1: pipette **50 µL** of citrated whole blood (alternatively pipette 25 µL of PRP and add 25 µL of diluted Reagent 1).
Add **150 µL** of diluted Reagent 1.
Homogenize using a vortex for 1 to 2 seconds.
- Tube T2: pipette **20 µL** Reagent 2a (anti GpIIIa MAb).
- Tube T3: pipette **20 µL** Reagent 2b (anti GpIb MAb).
- Tube T4: pipette **20 µL** Reagent 2c (anti GpIa MAb).
- Tube T5: pipette **40 µL** Reagent 3 (**vortex vial well before pipetting**).

6.2. Immuno-labelling of Samples

To <u>each</u> of tubes T2, T3 and T4:
- Add **20 µL** diluted sample (Tube T1).
- Homogenize the 3 tubes using a vortex for 1 to 2 seconds.
- Incubate all tubes at room temperature for **10 minutes**.

6.3. Fluorescent Staining

To <u>each</u> of tubes T2 to T5:
- Add **20 µL** Reagent 4.
- Homogenize the 4 tubes using a vortex for 1 to 2 seconds.
- Incubate at room temperature for **10 minutes**.
- Add **2 mL** diluted Reagent 1.
- Homogenize the 4 tubes using a vortex for 1 to 2 seconds.

Thus treated, the contents of all tubes may be stored for **2 hours** at **2-8 °C** before cytometric analysis.

6.4. Cytometric analysis

Refer to the Operator's Manual of the cytometer for instructions on how to perform cytometric readings.

The selected Mean Fluorescence Intensity (MFI) statistic is the geometric mean, Mn(x) or GeoMean.

Notes : the Beckman Coulter softwares Expo™ 32, CXP and RXP have an option baseline offset. This should be set OFF.

Vortex each tube before analysis.

- **Calibration analysis : Tube T5 (Figs 1)**

Create a FS LOG vs SS LOG cytogram. Add a discriminator on FS Log to minimize the artefactual background. Set up a gate "CAL" around the main single bead population (Fig. 1a).
Create a FL1 LOG histogram gated by the "CAL" window.
For optimum analysis conditions, the peak of the third bead fluorescence intensity (FL1) must be set at the beginning of the fourth decade.
To achieve this adjust the FL1 PMT voltage.
The "A" cursor must include the first channel.
For a correct analysis, at least 8,000 beads must be gated on the window "CAL".
Note the MFI for each of 3 fluorescence peaks (Fig. 1b : A, B and C cursors) corresponding to the 3 calibration beads.

Fig. 1a : Calibration cytogram

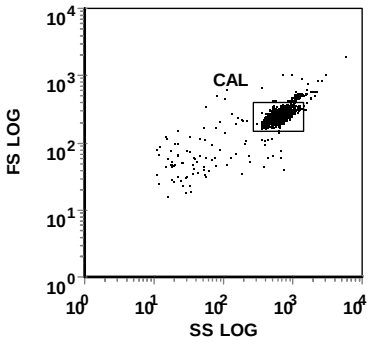
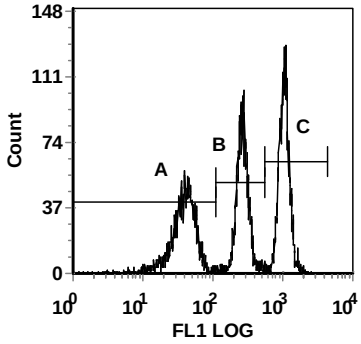


Fig. 1b : Cursor settings in gated fluorescence histogram



- **Sample analysis: tubes T2 to T4 (Figs 2)**

Do not change the acquisition procedure for FL1 (PMTv).
On the FS LOG x SS LOG cytogram (Fig. 2a) platelets are isolated from other whole blood cells by an analysis region " PLT ".
Check that the discriminator setting does not cut the platelet cloud.
Analyse at least 3,000 events on the window "PLT".
In the corresponding gated FL1 LOG histogram, note the mean fluorescence intensity of the positive peak of each assay (Fig. 2b).

Fig. 2a : Whole blood cytogram and platelet window gating

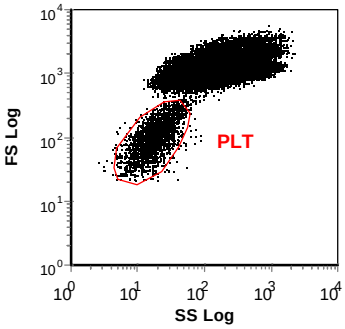


Fig. 2b : CD61 immuno-labelling, cursor settings

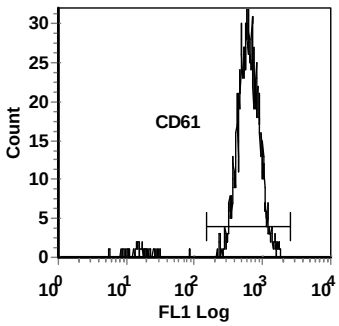


Fig. 2c : CD42b immuno-labelling, cursor settings

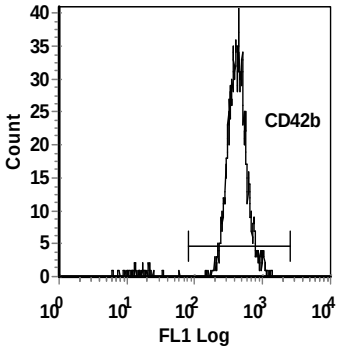
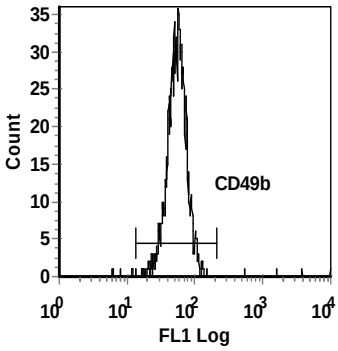


Fig. 2d : CD49b immuno-labelling, cursor settings



7 RESULTS

Computer data analysis or graphic data analysis.

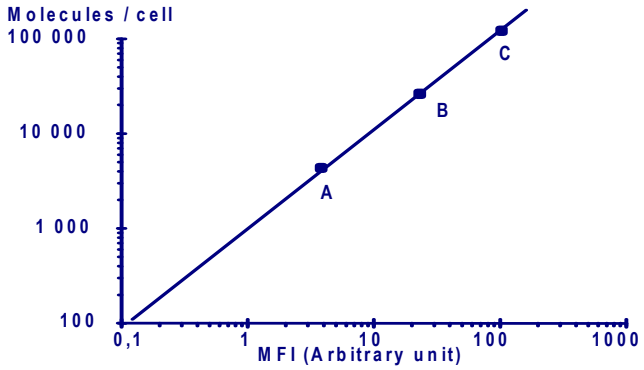
7.1. Computer data analysis:

The result treatment is easily performed using a calculation template available upon request from the BioCytex technical department.

7.2 Graphic data analysis :

Plot the LOG_{10} of the MFI calibration values for the 3 calibration beads on the abscissa (x-axis), and their corresponding LOG_{10} number of monoclonal antibody molecules as indicated on the calibration flyer, on the ordinate (y-axis).
Draw the optimal calibration curve of the type $LOG_{10}(ABC) = a \times LOG_{10}(MFI) + b$. Interpolate the LOG_{10} of the MFI values of the sample tubes on the calibration curve and read directly their corresponding numbers of bound Mab molecules (ABC : Antibody Binding Capacity).

Example of calibration curve:



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