

## PLATELET Gp Screen

Kit for platelet glycoprotein quantitation

Kit for 10 determinations

Ref.7008



**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 ( $\times 10^3$ )

### 3 REAGENTS

- **Reagent 1:** 1 x 15 mL vial, diluent, 10 fold concentrated.
- **Reagent 2a:** 1 x 200  $\mu$ L vial, anti GpIIIa MAb (CD61).
- **Reagent 2b:** 1 x 200  $\mu$ L vial, anti GpIb MAb (CD42b).
- **Reagent 2c:** 1 x 200  $\mu$ L vial, anti GpIa MAb (CD49b).
- **Reagent 3:** 1 x 400  $\mu$ L 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  $\mu$ L vial, staining reagent, polyclonal antibody anti mouse IgG-FITC.

#### WARNING

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

### 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 undiluted 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  $\mu$ L) 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  $\mu$ L** of citrated whole blood. Alternatively pipette 25  $\mu$ L of PRP and add 25  $\mu$ L of diluted Reagent 1 to obtain a 1:2 diluted PRP.

Add **150  $\mu$ L** of diluted Reagent 1.

Homogenize using a vortex for 1 to 2 seconds.

- Tube T2: pipette **20  $\mu$ L** Reagent 2a (anti GpIIIa MAb).
- Tube T3: pipette **20  $\mu$ L** Reagent 2b (anti GpIb MAb).
- Tube T4: pipette **20  $\mu$ L** Reagent 2c (anti GpIa MAb).
- Tube T5: pipette **40  $\mu$ L** Reagent 3 (**vortex vial well before pipetting**).

#### 6.2. Immuno-labelling of Samples

To each of tubes T2, T3 and T4 :

- Add **20  $\mu$ L** 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 each of tubes T2 to T5 :

- Add **20  $\mu$ L** 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.

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) statistics is the geometric mean (Mn (x) or GeoMean depending upon the cytometer). Vortex each tube before analysis.

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

Create a FS LOG vs SS LOG cytogram.

Add a discriminator on FS to minimize the artefactual background. Set up a gate ("A") around the main bead population (Fig. 1a).

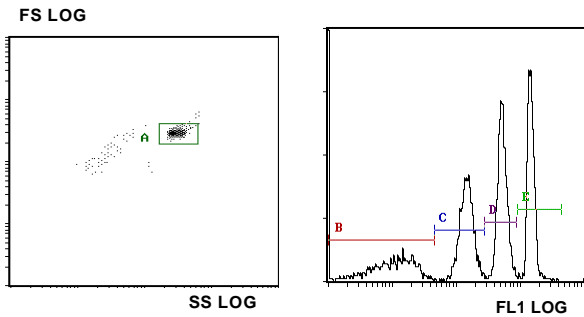
Create a FL1 LOG gated by the "A" region.

Note the mean fluorescence intensity (MFI) for each of the 4 fluorescence peaks (Fig. 1b : B, C, D and E cursors) corresponding 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 :** Test calibration cytogram

**Fig.1b :** Cursor settings in gated fluorescence histogram



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

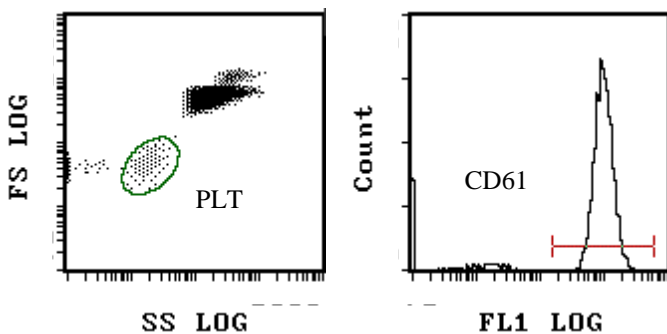
**Do not change the acquisition procedure for FL1 (PMT, V).**

On the FS LOG x SS LOG cytogram (Fig. 2a) platelets are isolated from other whole blood cells by an analysis region "PLT".

In the corresponding gated FL1 LOG histogram, note the mean fluorescence intensity of the positive peak of each assay (Figs. 2b, 2c and 2d).

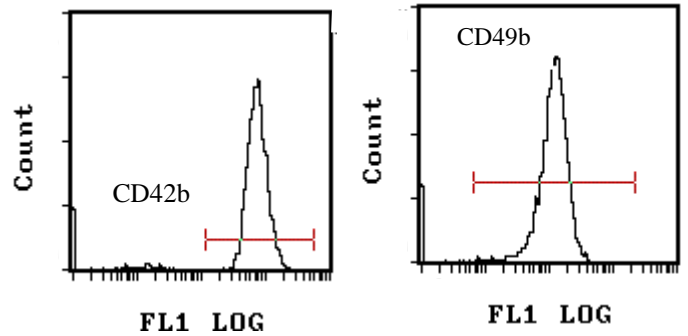
**Fig. 2a :** Whole blood cytogram and platelet window gating

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



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

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



## 7 RESULTS

Depending on the instrument used:

If the MFI values (Mean Fluorescence Intensity) are expressed as linearized values, use a log-log graph paper.

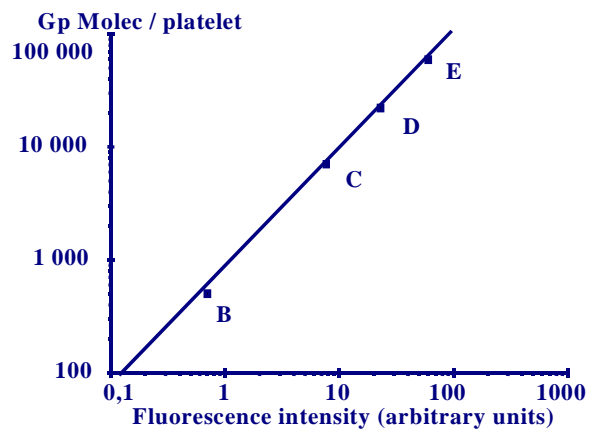
If the MFI values are obtained as channel numbers, use a semi-log graph paper.

Using a log-log or a semi-log graph paper, plot the MFI calibration values (tube T5) on the abscissa (x-axis) and their corresponding number of monoclonal antibody molecules (as indicated in the calibration flyer) on the ordinate (y-axis).

Draw the calibration curve.

Interpolate the MFI values of the tubes T2 to T4 on the calibration curve and read off directly the corresponding number of molecules.

**Example of calibration curve:**



## REFERENCES

- 1- Schlossman *et al.* eds 1995: "Leucocyte Typing V, White Cell Differentiation Antigens", Oxford Univ. Press, pp 1309-1315, 1615-1616.
- 2- Schmitz G. *et al.* " European working group on clinical cell analysis: Consensus protocol for the flow cytometric characterisation of platelet function" *Thromb.Haemost.* 1998; 79:885-896.

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