Determination of the saturating concentration of a monoclonal antibody to be used in the PLATELET Calibrator kit

Recommended protocol

Definition of the saturating concentration of a monoclonal antibody (MAb) : MAb concentration which enables to saturate all accessible antigenic sites at the surface of the cells of interest.

A / Setup of the dilutions of the specific monoclonal antibody :

For one specific MAb to be tested, prepare 6 dilutions according to the following table :

Dilutions	Volume of the specific MAb	Volume of diluted R1	Concentration of the specific MAb
D1	300 µL		20 µg/mL
D2	150 µL of D1	+ 150 µL	10 µg/mL
D3	150 µL of D2	+ 150 µL	5 µg/mL
D4	150 µL of D3	+ 150 µL	2.5 µg/mL
D5	150 µL of D4	+ 150 µL	1.25 µg/mL
D6	150 µL of D5	+ 150 µL	0.6 µg/mL

B/ Setup of the staining reagent (R4)

For one specific MAb: mix **70 µL** of Reagent 4 and **630 µL** of diluted Reagent 1. Homogenize using a vortex for 1 to 2 seconds.

C/ Protocol

Label 6 test tubes T1 to T6.
Prepare a platelet rich plasma (PRP) from a citrated whole blood sample (trisodium citrate 0.109 M or 0.129 M using a ratio 9:1 volumes) or drawn on CTAD / Diatube®H (Becton-Dickinson).
PRP preparation: Centrifuge the blood sample, at **170g** for **15 minutes** at room temperature.
Collect the PRP in a test tube and homogenize the tube by inverting it gently up and down.

To each tube T1 to T6:
Pipette **5 µL** of PRP and add **100 µL** of the dilutions prepared according to paragraph A.
Homogenize the tubes using a vortex for 1 to 2 seconds.
Incubate the tubes at room temperature (18-25 °C) for **15 minutes.**
Perform one washing :
- Add to tubes T1 to T6, **3 mL** of diluted Reagent 1.
- Centrifuge the tubes for **5 minutes** at **1900 g.**
- Discard the supernatant.

Add **100 µL** of diluted Reagent 4 prepared according to paragraph B.
Homogenize the tubes using a vortex for 1 to 2 seconds.
Incubate the tubes at room temperature for **15 minutes**.
Perform 2 more washings as described above.
Add **500 µL** of diluted Reagent 1.

D/ Cytometric analysis

Vortex each tube before analysis.
Refer to the Operator's Manual of the cytometer for instructions on how to perform cytometric readings.

Create a FS LOG vs SS LOG cytogram.
Add a discriminator on FS to minimize the artefactual background.
Isolate the platelet population from other whole blood cells by an analysis region "PLT" (fig.a).
Create a FL_1 LOG histogram gated by the "PLT" region.
Note the mean fluorescence intensity (MFI) for each dilution of the specific MAb (fig.b).

Fig.a : Platelet region gating

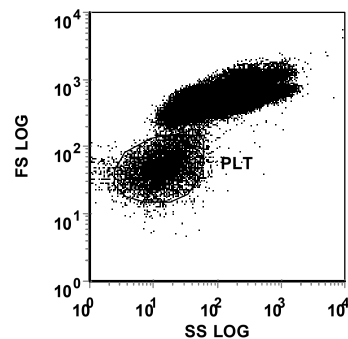
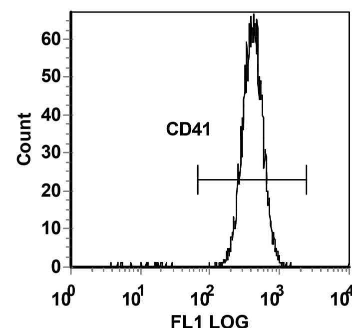
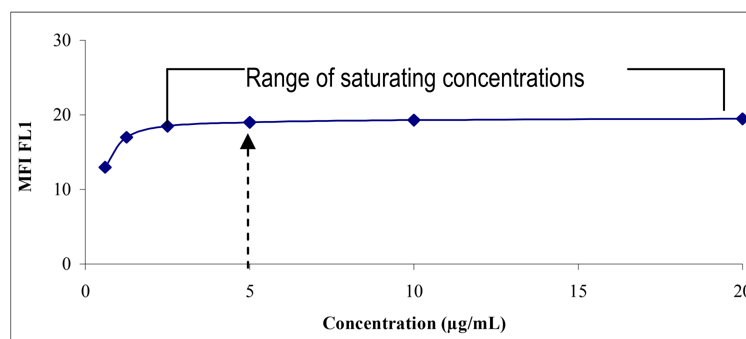


Fig.b : CD41 Immuno-labelling



E/ Results

With a calculation software, draw the saturation curve converting the MFI to the concentration of the antibody.
To achieve this plot the 6 tested concentrations on the abscissa (X axis) and their corresponding MFI values on the ordinate (Y axis).



The saturation curve presents a plateau corresponding to the range of saturating concentrations of the MAb.
Check that the concentration of **5µg/mL** give a MFI situated on the plateau of the saturation curve. If this is confirmed, the MAb can be used in the protocol of the PLATELET Calibrator at the concentration of **10µg/mL**.

Note: The use of specific MAb not saturating at a concentration ≤ 5µg/mL in the kit protocol will lead to incorrect results.

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