MP-Count Beads

For microparticle absolute count by flow cytometry

Vial for 100 Tests **Ref. 7804**

BioCytex

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

1 INTRODUCTION

Biological microparticles ("MP") are cellular vesicles of heterogeneous size ranging from 0.1 to 1 µm. In order to get an absolute count of microparticles by flow cytometry, the use of small sized counting beads is a pre-requisite. Thus MP-Count Beads are the appropriate beads for this application.
They fit with the settings of MP analysis and their small size (3 µm diameter) avoids artefactual loss and problems of sedimentation that are often encountered with the use of bigger beads. (2, 3)

2 PRINCIPLE

Once the sample (platelet free plasma PFP or purified MP) is stained, a volume of MP-Count Beads identical to the volume of sample is added to the analysis tube.
Analysing MP-Count Beads of known concentration spiked into each sample allows calculating the concentration of MP (MP/µL) in the sample of interest using the following formula:

$$MP/\mu L = \frac{\text{Nb MP counted} \times [\text{MP-Count beads}]}{\text{Nb MP-Count Beads counted}}$$

3 REAGENT PROVIDED

1 vial of 3 mL of beads.
MP-Count Beads is a suspension of 3 µm FL2 / PE fluorescent beads with known concentration (see vial label indicating the bead concentration).

WARNING
- Follow the conventional laboratory practices.
- Biological samples must be considered as potentially infectious.
- Follow the appropriate reglementation for waste disposal.

4 MATERIAL REQUIRED BUT NOT PROVIDED

- Cytometer with blue (488 nm) or yellow (561 nm) laser.
- Stirring machine type Vortex.
- Rotary stirring machine
- Cytometry tubes (similar to those recommended for MP analysis, i.e. polypropylene tubes, with low dust and protein adsorption).
- Megamix (ref. 7801), Megamix-Plus FSC (ref. 7802) or Megamix-Plus SSC (ref. 7803) for initial cytometer settings.
- Reagents for MP staining (e.g. MAb-PE, Annexin V-FITC, binding buffer/ calcium buffer).

5 REAGENT PREPARATION AND STORAGE

Unopened vial remains stable until expiration date printed on the label when stored at 2-8°C. Do not freeze.
The reagent is ready for use.

Allow the reagent to stay at room temperature for at least 30 minutes before use.

The reagent can be placed under gentle rotary stirring or vortexed for 10 seconds just before use.

Stability after opening: 6 months at 2-8°C when free of contamination.

6 PROCEDURE

The reagent must be at room temperature for the procedure.

6.1 Sample preparation

Note: it is recommended to use the same pipette for adding both the sample and the MP-Count Beads, a potential volume error being thus neutralized by the calculation formula.

The illustration below is provided only as an example, since FCM-based analysis protocols may widely vary (2).
For a PMP $PS^+/CD41^+$ absolute count on Gallios (Beckman-Coulter), starting from a PFP sample, the following protocol can be applied.

For each PFP staining, label a cytometry tube.

- Pipette 10 µL of CD41-PE (réf. 5112-PE100T) and 10 µL of Annexin V-FITC.
- Add 30 µL of PFP.
- Homogenize the tubes for 1 to 2 seconds using a vortex.
- Incubate for 30 minutes at room temperature in the dark.
- Add 500 µL of binding buffer (calcium buffer).
- Add **30 µL** of MP-Count Beads previously homogenized.

The MP-Count Beads vial can remain at room temperature for maximum 2 hours and must be replaced at 2-8°C thenafter.

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

6.2 Cytometric analysis

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

Vortex the tube for 1 to 2 seconds before analysis.

- Proceed to the acquisition of Megamix bead tube before each MP series (cf. inserts of products 7801, 7802 or 7803) in order to set the instrument. In the example below, Megamix-Plus FSC is analysed on Gallios (Figs 1a and 1b).

Fig.1a : Megamix-Plus FSC beads distribution (FS threshold)

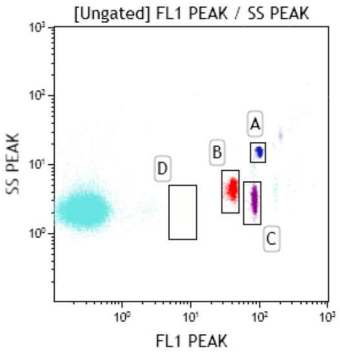
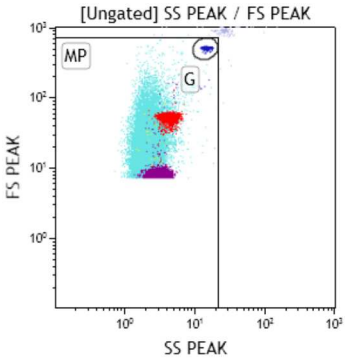


Fig.1b : MP gate setting

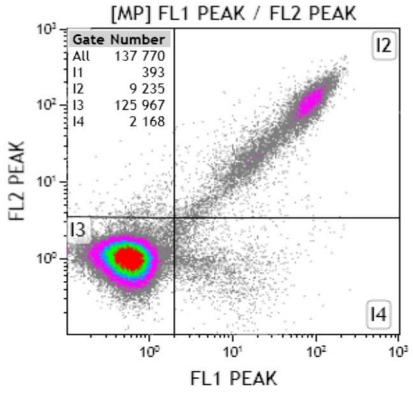


Region A : 0.9 µm beads
Region B : 0.5 µm beads
Region C : 0.3 µm beads
Region D : 0.1 µm beads

- Then proceed to the acquisition of the stained sample **within the same settings than the Megamix-Plus FSC ones** (Fig.2).

The following example shows a PMP staining (CD41-PE/Ann. V-FITC) with optimized fluorescent compensations.
- Note the number of MP present within the region I2. In this example, n = 9,235 events.

Fig.2 : Density plot of PMP gated on "MP gate"



- Create a FL2 Log x SS Log cytogram (Fig. 3a).
- Set PMT FL2 and PMT SS in order to have the MP-Count Beads appearing in the middle of the 4th decades both in SS and FL2 scales.
- Set up a 'MP-Count' region around the dense cloud of the beads avoiding the doublets.
- Create a FL2 LOG x Count histogram gated on the 'MP-Count' region.
- Set up a cursor K and note the number of detected MP-Count Beads (Fig.3b). In this example, n = 1,024.

- Analyse at **low speed** (around 10-15 µL/mn) for a duration corresponding to a **minimum of 500 MP-Count Beads** counted (**1 minute minimum** is recommended).

It is recommended to follow the stability of the flow during the analysis using an histogram Time x Count (Fig.3 c).

Fig.3a : MP-Count Beads gating

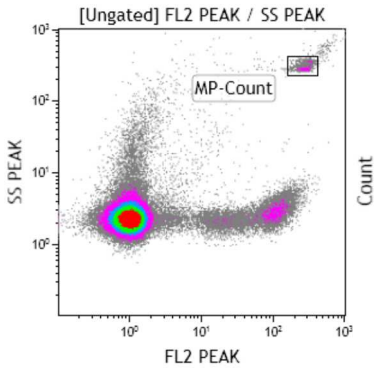


Fig.3b : FL2 MP-Count Beads distribution

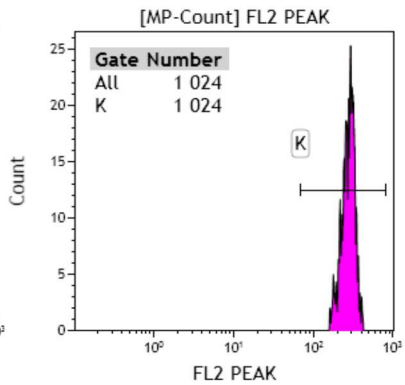
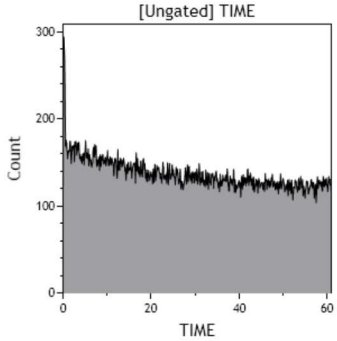


Fig.3c : Total events analysed overtime



The MP count can be affected by a problem of flow.
It is thus advised to follow its stability during acquisition. In case of a flow problem, it is recommended to proceed to a new acquisition of the sample. If not possible, one may also gate out the problematic part of the analysis based on Time x Count histogram.

The absolute MP count within this sample is then calculated as follows :

$$MP/\mu L = \frac{\text{Nb MP counted} \times [\text{MP-Count Beads}]}{\text{Nb MP-Count Beads counted}}$$

MP/µL = (9,235 x 2,200)/1,024 = 19,841 PMP / µL
(2,200 being the MP-Count Beads concentration of the batch used here).

Note : the addition of a fixative (such as paraformaldehyde, PFA) to the MP cytometric tube may lead to a FL2/PE decrease of the MP-Count Beads.

REFERENCES

1) Robert S et al, Artherioscler Thromb Vasc Biol, 2012; 32: 1054-58.
2) Poncelet P et al, Transfus Apher Sci. 2015; 53(2):110-26.
3) Poncelet P et al, Cytometry A. 2016; 89(2):148-58.

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