



# Megamix

Beads for cytometer settings in microparticle analysis

Kit for 50 Tests

Ref. 7801

## For Research use only

### 1 INTRODUCTION

Biological microparticles ("MP") are cellular vesicles of heterogeneous size ranging from 0.1 to 1  $\mu\text{m}$ . Due to their small size, MP analysis requires working conditions close to the size sensitivity limit of the cytometers. Standardization of MP count requires to master this limit located at 0.5  $\mu\text{m}$  for an optimal compromise between MP analysis and background exclusion.

Megamix reagent allows to standardize the set-up of MP analysis region (0.5-1  $\mu\text{m}$ ) and guarantee the stability of the settings.

### 2 PRINCIPLE

Megamix is a mix of fluorescent beads of varied diameters to cover the MP (0.5 and 0.9  $\mu\text{m}$ ) and platelet size ranges (0.9 and 3  $\mu\text{m}$ ).

This bead acquisition according to the following procedure allows the settings of the cytometer to study MP within a constant size region and to get reproducible MP counts.

Megamix also allows to transfer the protocol and the settings from one cytometer to another and to guarantee the stability over time.

Limitation: some cytometers from previous generation do not feature enough FS resolution to be used for standardized MP counts. This will lead to a bad resolution between 0.5 and 0.9  $\mu\text{m}$  beads (no separation observed in Fig.4).

### 3 REAGENT PROVIDED

1 vial of 25 mL of beads.

Megamix is a mix of beads from chosen diameter : 0.5  $\mu\text{m}$ , 0.9  $\mu\text{m}$  and 3  $\mu\text{m}$ .

### 4 MATERIAL REQUIRED BUT NOT PROVIDED

- Cytometer.
- Stirring machine type vortex.
- Cytometry tubes (similar to those used for MP analysis, low dust).
- Adjustable pipettes with disposable tips (500  $\mu\text{L}$ ).

### 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.

**Resuspend this reagent by vortexing for 10 seconds before use.**

Stability after opening : until expiration date printed on the label at 2-8°C when free contamination.

### WARNING

The reagent contains sodium azide as a preservative. Reagent 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.

### 6 PROCEDURE

The reagent must be at room temperature during the procedure.

We recommend to run Megamix beads before each series of MP analysis.

#### 6.1 Bead preparation

Take a cytometry tube.

Pipette into it 500  $\mu\text{L}$  of Megamix reagent after shaking vigorously the reagent vial for at least 10 seconds by vortexing.

#### 6.2 Cytometer settings

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

Vortex the tube before analysis.

For the cytometric acquisition protocol, create :

- one cytogram FL1 Log x SS Log (density plot)
- one cytogram SS Log x FS Log
- one cytogram FL1 Log x FL2 Log
- one histogram FS Log x count
- one histogram FL3 Log x count

Proceed to the following settings :

- Gains for FS and SS : high
- Time of analysis : at least 1 minute
- Speed of analysis : the lowest available.

The following analysis has been performed on Cytomics FC500 cytometer (Beckman Coulter). The suggested settings below apply for this type of instrument :

- gain SS at 10
- gain FS at 10
- PMTv FS between 150 and 200 volts.

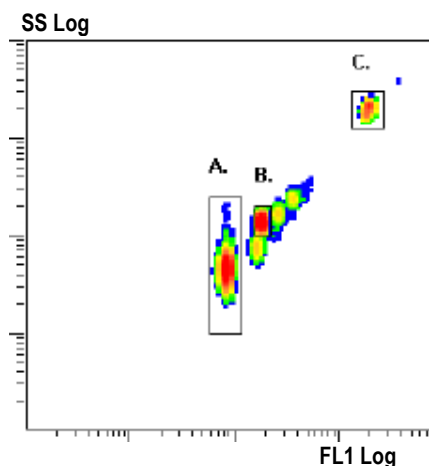
##### 6.2.1 Settings of FL1 PMT and SS PMT (Fig.1)

- Proceed to acquisition of Megamix bead tube.
- Add a **discriminator on FL1, threshold = 1** to only analyze FL1+ events and to eliminate the contaminants (background of the instrument).

On the FL1 Log x SS Log cytogram:

- Create 3 regions "A", "B" and "C" around each of the singlet bead populations. Take care to gate region "B" around the dense cloud of 0.9  $\mu\text{m}$  beads corresponding to the singlets.
- Adjust the FL1 PMT voltage in order to set the 3  $\mu\text{m}$  bead cloud at the beginning of the 4<sup>th</sup> decade (MFI ~ 200 a.u.)
- Adjust the SS PMT voltage in order to set the 3  $\mu\text{m}$  bead cloud at the beginning of the 4<sup>th</sup> decade (MFI ~ 200 a.u.).

Fig.1 : Settings of FL1 PMT and SS PMT



Region A : beads of 0.5  $\mu\text{m}$

Region B : beads of 0.9  $\mu\text{m}$

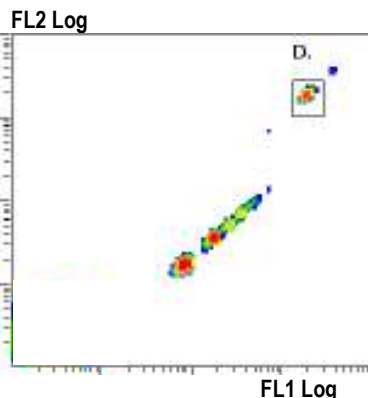
Region C : beads of 3  $\mu\text{m}$

### 6.2.2 Settings of FL2 PMT (Fig.2)

On the FL1 Log x FL2 Log cytogram :

- Create a region "D" around the 3 μm singlet bead cloud.
- Adjust the FL2 PMT voltage in order to set the 3 μm bead cloud at the beginning of the 4<sup>th</sup> decade (MFI ~ 200 a.u.).

Fig.2 : Settings of FL2 PMT

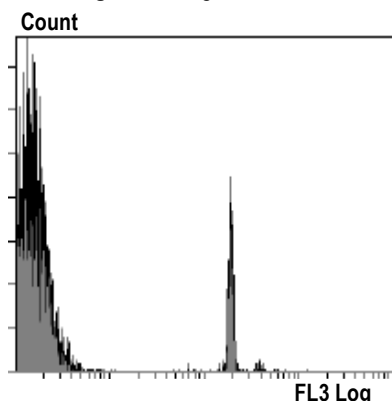


### 6.2.3 Settings of FL3 PMT (Fig.3)

On the FL3 Log x Count histogram :

- Adjust the FL3 PMT voltage in order to set the 3 μm bead peak at the end of the 2<sup>nd</sup> decade or at beginning of the 3<sup>rd</sup> decade.

Fig.3 : Settings of FL3 PMT

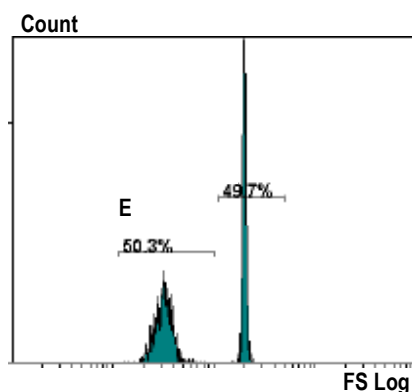


### 6.2.4 Settings of FS PMT (Fig.4)

- Switch discriminator on FS, threshold = 1.

- On the FS Log x Count histogram gated on 0.5 μm and 0.9 μm bead singlets (gate "A or B"), adjust FS PMT voltage in order to obtain 50% of 0.5 μm bead (region E). To decrease the 0.5 μm percentage, diminish FS PMT voltage, and conversely. A range of 48 to 52% is acceptable.

Fig.4 : Settings of FS PMT



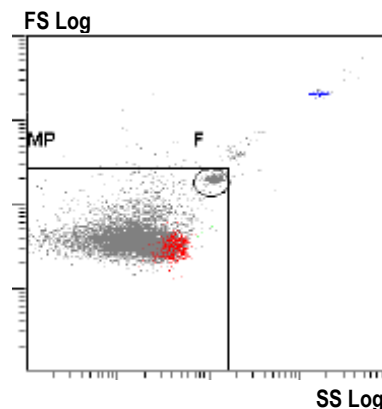
### 6.2.5 MP analysis region determination (Fig.5)

The MP analysis region is defined as follows :

- the lower side is defined by the threshold (here FS = 1) allowing acquisition of events of at least 0.5 μm (settings already performed at paragraph 6.2.4).
- the upper side is the end of 0.9 μm bead cloud.

To achieve this, on the SS Log x FS Log cytogram, create an autogate "F" (maximum sensitivity) around the 0.9 μm bead cloud and create a "MP gate" tangent to the upper and right sides of the autogate "F" (Fig.5).

Fig.5 : MP gate



### 6.3 Routine Megamix analysis

Acquisition protocol being created (cf paragraph 6.2) proceed to acquisition of Megamix bead tube.

- Check that the beads are located within the pre-existing regions (A, B and C). If not, adjust FL1 PMT and/or SS PMT to achieve it.
- Check on FS Log x Count histogram that the indicated 0.5 μm bead percentage is close to 50% (region E : 48-52%). If not, adjust FS PMT to achieve it.
- Check that the "MP gate" is still correctly set. If not, adjust its position.

After these 3 checkings, cytometer settings are optimal and allow a standardized acquisition of MP.

### 6.4 Example of platelet MP staining : PMP (Figs 6)

Following an AnnexinV-FITC/CD41-PE staining and appropriate fluorescence compensation settings, here is an example of PMP analysis.

Fig.6a : TMP (total MP) gated on "MP gate"

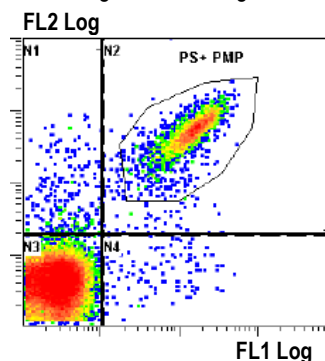


Fig.6b : PS+ PMP size distribution

