Calibration kit HemoStep

REF	Σ	
IMS1511	10 test	CE IVD

1. INTENDED USE

The intended use of this kit is for the calibration of the standard curve of the HemoStep kit. Calibration ensures the proper performance of the kit and prevents small variations that may occur in the flow cytometer over time affecting the results. The kit consist of a set of microspheres or beads coated with different haemoglobin (Hb) concentrations corresponding to the top and bottom of the standard curve as well as an anti Hb antibody conjugated with phycoerythrin (PE).

This combination allows to user to obtain as fluorescen signal value. via flow cytometry analysis and use this fluorescence value tu recalibrate the standard curve if necessary, thus avoiding the need to build a new standard curve, saving time and reagents.

2. COMPONENTS

A) **MICROSPHERES:** Two distinct populations of 6 µm magnetic polystyrene microspheres (top and bottom) with an internal fluorescence pattern different from capture beads. Supplied in lyophilized form in a single flow cytometry tube with a cap. Each tube contains 2000 microspheres (1000 of each population).

B) **ANTIBODY:** 0.1 ml of phycoerythrin (PE)-conjugated detection antibody (10 µl/test). Supplied in a 0.1 ml vial, ready-to-use concentration, in buffered aqueous solution containing protein stabilizer and 0.09% sodium azide (NaN_3) .

3. STORE AND HANDLING CONDITIONS

Store refrigerated between +2 and +8°C.

DO NOT FREEZE.

4. RECOMENDACIONES Y ADVERTENCIAS

- FOR IN VITRO DIAGNOSTIC USE. For professional use only.
- Only for qualified laboratory personnel.
- Kit components contain KATHON™ or sodium azide (NaN3). Compounds should be dissolved with tap water before disposal. These conditions are recommended to avoid deposits in pipes. Material Safety Data Sheet (MSDS) available on the website www.immunostep.com.
- Before starting the analysis, read the instructions carefully. Deviations from the recommended procedures may invalidate the assay results. Do not substitute or mix Immunostep kit reagents with reagents from other manufacturers.
- Before acquiring calibration beads, it is necessary to ensure that the flow cytometer settings and their compensation are appropriate.
- Keep kit components away from direct light exposure during the protocol. Fluorescently conjugated antibodies and microspheres are sensitive to light.
- Reagents must not be used if the packaging shows clear evidence of deterioration.
- Wear personal protective equipment for sample handling. Wash hands properly after handling specimens. All procedures should be carried out in accordance with approved safety standards.
- For use only in combination with the HemoStep kit (Ref: IMS1510).

5. REAGENT PREPARATION

Equilibrate reagents at +18 °C to +24 °C (room temperature) for 30 minutes prior to use.

6. ASSAY PROCEDURE

1. Reconstitute beads by adding 50 µl of incubation buffer to the calibration tubes and allow to equilibrate for 2 minutes.
2. Add 10 µl/test of the detection antibody to the calibration tubes and incubate for 15 minutes at room temperature, in the dark, with agitation.
3. After incubation, wash with 1 ml of 1X wash buffer, vortex, and incubate on the magnetic rack for 5 minutes (The beads can also be collected by centrifugation at 2500xg for 5 minutes).
4. After 5 minutes, discard the supernatant and resuspend the tubes in 200 µl of wash buffer.
5. Acquire on the flow cytometer.

7. ANALYSIS STRATEGY FOR CALIBRATION BEADS

As in the previous point, a first step of selecting the bead population on the FSC-H/FSC-A dot plot to remove doublets is recommended (A), followed by a selection of the bead population on the SSC-A/FSC-A dot plot to remove residual dirt and reduce background (B), allowing the correct identification of the two calibration bead populations on a dot plot for any of the following channels PerCP/APC, PerCP-Cy5/APC or PerCP-Cy5. 5 / APC (C).
The two calibration bead populations show different MFI values in the PE fluorescence channel. These MFI values, identified as "bottom" and "top," correspond to parameters (a) and (d) of the standard curve generated with the HemoStep kit.

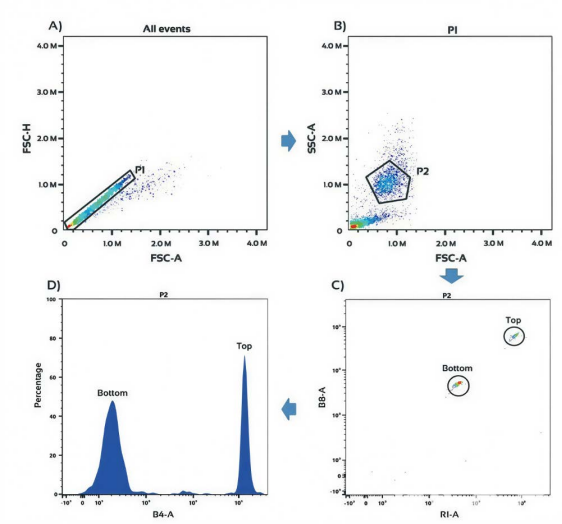

Figure 1: Analysis strategy for selecting the two calibration bead populations in FSC-H/FSC-A (A); SSC/FSC (B); PerCP/APC (C); and PE histogram (D).

Thanks to the use of calibration beads, it will not be necessary to generate a standard curve for each assay day. A new standard curve will only need to be generated in the following cases:

- When using a reagent kit from a new lot.
- When the Flow cytometer configuration has been change.

8. EXPLANATION OF SYMBOLS

Σ	The content is sufficient for <n> analysis
REF	Product reference
CE	CE labeling
IVD	In vitro diagnostic device
	Manufacturer

9. MANUFACTURER

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