ab322700 – Compensation Beads Kit, Goat anti-Mouse Ig (3.0-3.4 µm)

Binds to all mouse and rat isotypes, most hamster isotypes and rabbit polyclonal IgG to generate compensation standards for flow cytometry studies.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab322700

Storage and Stability:

The entire kit may be stored at +4°C for up to 1 year from the date of shipment. Do not freeze and protect the kit from light. For prepared reagent storage, see table below.

Materials Supplied

Item	Quantity	Storage Condition
Compensation beads Gt anti-Ms Ig(H&L) Particles, High Binding, 3.0-3.4 um, 5 mL	1 x 5 mL	+4°C
Compensation beads Gt anti-Ms Ig(H&L) Particles, Blank, 3.0-3.4 um, 5 mL	1 x 5 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this kit:

- Fluorochrome conjugated antibodies.
- Flow cytometer.
- Adjustable 50 µL-1 mL pipettes for reagent preparation.
- Microcentrifuge tubes.
- Staining buffer – 1 x PBS (pH 7.4) with 0.02% sodium azide and 0.2%BSA
- Vortex mixer
- Microcentrifuge
- Sonicator
- Computer and software for fluorescence histogram data analysis.

Reagent Preparation

- Before use resuspend by vortexing. To achieve optimum particle suspension, sonicate the reagent after vortex mixing.
- Equilibrate the beads to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

Assay Procedure

1. Remove the beads from the refrigerator and allow them to come to room temperature and briefly vortex.

2. Add one drop (~50 µL) of negative beads and one drop (~50 µL) of positive beads to each tube.

3. Label microcentrifuge tubes for each of the single colour antibody conjugates to be used.

4. Add antibody conjugates to each tube at the concentration used in your application and vortex immediately

5. Incubate all tubes at room temperature in the dark for 20 minutes

6. Wash the beads by adding 1mL of Staining Buffer to each tube, vortex and centrifuge at 300 x G for 5 minutes.

7. Decant and resuspend in 600µL Staining Buffer with a brief vortex.

8. Analyze on a flow cytometer using an acquisition protocol with FS (Lin)vs. SS (Lin) dot plot and either single color histograms or dual color dot plots for each relevant fluorescent channel.

    a. *Note: Verify that the discriminator is low enough to detect the singlet bead population.*

9. Using cytometer settings optimized for your application, run each of the stained bead samples and ensure that the positive signals are on scale.

10. Generate a compensation matrix using acquisition and/or analysis software

    a. *Note: If large amounts of doublets and triplets are visible in the FS vs SS histogram, sonicate the stained sample for 60 seconds and reanalyze .*

    We recommend that you assay all standards, controls and samples in duplicate. Prepare all reagents as directed in the previous section. Dye & bead concentrations may be further optimized for best results. Centrifugation force and time may need to be increased if needed.

For technical support contact information, visit: www.abcam.com/contactus

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