Technical Data Sheet

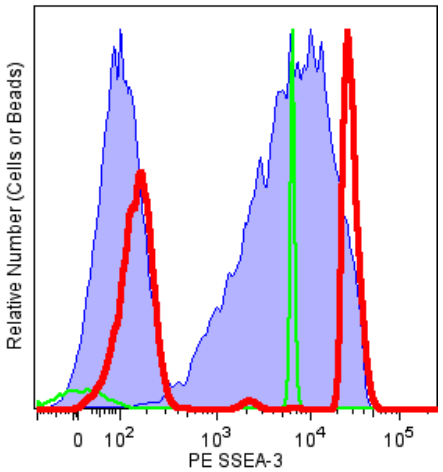
Anti-Rat Ig, κ/Negative Control (BSA) Compensation Plus (7.5 µm) Particles Set

Product Information

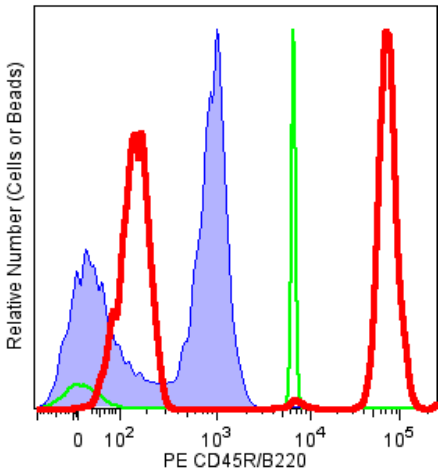
Material Number:	**560499**
Component:	**51-9006227**
Description:	Negative Control (PBS with 1% BSA)
Size:	6.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component:	**51-9006320**
Description:	Anti-Rat Ig, κ
Size:	6.0 ml (1 ea)
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD™ CompBead Plus Anti-Rat Ig, κ beads are polystyrene particles used to establish fluorescence compensation settings for multicolor flow cytometric analyses. The kit provides two types of particles, the BD™ CompBead Plus Anti-Rat Ig, κ particles, which bind any rat κ light chain-bearing immunoglobulin, and the BD™ CompBead Plus Negative Control (BSA) particles, which have no binding capacity. When mixed together with a fluorochrome-conjugated rat antibody, the BD™ CompBead Plus provides distinct positive and negative (background fluorescence) stained populations which can be used to set compensation levels manually or using instrument set-up software. Since the compensation adjustments are made using the same fluorochrome-labeled antibody to be used in the experiment, this method allows the investigator to more accurately establish compensation corrections for spectral overlap for any combination of fluorochrome-labeled antibodies (without having to use valuable tissue samples or hard-dyed beads with potentially mismatched fluorescence spectra). Use of the BD™ CompBead Plus is highly recommended for use in all experiments using tandem dye (i.e., PE-Cy™7, APC-Cy™7, etc.) conjugates, which may have distinct spectral characteristics for each conjugate.



H9 human embryonic stem cells (WiCell, Madison, WI) (hESCs), BD™ CompBeads (Cat. No. 552843), and BD™ CompBead Plus were stained with PE rat anti-SSEA-3 (Cat. No. 560237) and analyzed on a flow cytometer. Both unstained and stained hESCs are shown in blue, negative and stained BD™ CompBeads in green, and negative and stained BD™ CompBead Plus in red. Flow cytometry was performed on a BD LSR™ II flow cytometry system.



BALB/c splenocytes, BD™ CompBeads (Cat. No. 552843), and BD™ CompBead Plus were stained with PE rat anti-mouse CD45R/B220 (Cat. No. 553089) and analyzed on a flow cytometer. Stained splenocytes are shown in blue, negative and stained BD™ CompBeads in green, and negative and stained BD™ CompBead Plus in red. Flow cytometry was performed on a BD LSR™ II flow cytometry system.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

Note: BD Horizon™ V500 and AmCyan conjugated reagents can show significant differences in emission spectrum on stained cells and when captured on BD™ CompBeads. Thus, spillover values for these dyes evaluated with BD™ CompBeads may not provide correct compensation for cells. Therefore, single stained cellular controls are recommended to set up compensation for AmCyan and BD Horizon™ V500 reagents. BD Horizon™ V500-C has been modified to minimize these spectral differences and BD™ CompBeads may be used to determine spillover values for RUO antibodies conjugated to BD Horizon™ V500-C.

This BD™ CompBead Plus Set has been tested with rat Ig antibodies conjugated to various fluorochromes and analyzed using a BD FACS brand flow cytometer to ensure specificity and reactivity of the particles. See the specific instructions below on the use of the BD™ CompBead Plus Set:

1. Vortex BD™ CompBead Plus thoroughly before use.
2. Label a separate 12 x 75 mm sample tube (BD Falcon™, Cat. No. 352008) for each flurochrome-conjugated rat Ig, κ antibody to be used on a given experiment.
3. Add 100 µl of staining buffer [e.g., BD Pharmingen™ Stain (FBS), Cat. No. 554656 or BD Pharmingen™ Stain (BSA), Cat. No. 554657] to each tube.
4. Add 1 full drop (approximately 60 µl) of the BD™ CompBead Plus Negative Control (BSA) and 1 drop of the BD™ CompBead Plus Anti-Rat Ig, κ beads to each tube and vortex.
5. Add 20 µl of each prediluted antibody stock (diluted to a concentration optimal for staining 10^6 cells) to be tested on a given experiment to the appropriately-labeled tube. (Make sure the antibody is deposited to the bead mixture, then vortex.)
6. Incubate 15 - 30 minutes at room temperature. Protect from exposure to direct light.
7. During the incubation of beads and antibody, set the flow cytometer instrument PMT voltage settings using the target tissue for the given experiment (eg, whole blood, splenocytes, etc). If you are unsure, use the BD™ CompBead Plus Negative Control (BSA) beads as your negative reference point and proceed.
8. Following the incubation step (see Step 6 above), add 2 ml staining buffer to each tube and pellet by centrifugation at 200 x g for 10 minutes.
9. Discard supernatant from each tube by careful vacuum aspiration using a fine-tip Pasteur pipette.
10. Resuspend bead pellet in each tube by adding 0.5 ml of staining buffer to each tube. Vortex thoroughly.
11. Run each tube separately on the flow cytometer. Gate on the singlet bead population based on FSC (forward-light scatter) and SSC (side-light scatter) characteristics.
12. Adjust flow rate to 200 - 300 events per second if possible.
13. Use the compensation tool in BD™ FACSDiva software to calculate the compensation for your experiment, or proceed to step 14 to manually adjust compensation values.
14. To manually set compensation values, create a dot plot for the given fluorochrome-conjugated antibody as appropriate [i.e., to set compensation for a fluorescein (FITC)-conjugated antibody, use an FL1 vs. FL2 dot plot].
15. Place a quadrant gate such that the negative bead population is in the lower left quadrant and the positive bead population is in the upper or lower right quadrant, and adjust the compensation values until the median fluorescence intensity (MFI) of each population (as shown in the quadrant stats window) is approximately equal (i.e., for FL2 -%FL1, the FL2 MFI of both bead populations should be approximately equal when properly compensated).
16. Repeat Steps 14 and 15 for other tubes, as necessary.
17. Proceed to acquiring the actual staining experiment.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)

Product Notices

1. Cy is a trademark of Amersham Biosciences Limited.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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