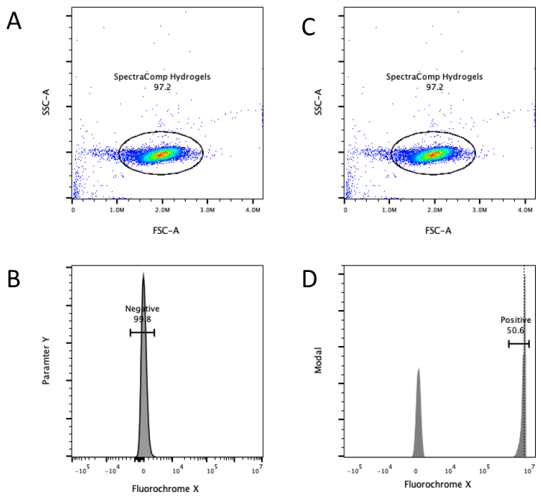
1. Technical Data Sheet

Summary	SpectraComp® compensation controls are state-of-the-art hydrogels that capture multiple antibody host species (mouse anti-human, mouse, rat, and hamster), and mimic the fluorescence spectra of stained cells
Application	SpectraComp® are intended as compensation controls to match the single staining performance of real cells. Staining the capture beads yields a positive fluorescence histogram that will aid in resolving the performance of the fluorophore; it will also serve as the basis for positive signal of a given fluorophore for compensation and/or spectral unmixing. **For Research Use Only. Not for use in diagnostic or therapeutic procedures.**
Materials	SpectraComp® are hydrogels that are suspended in aqueous solution and are packaged in a convenient dropper bottle. Each drop contains approximately 1 x10^5 beads.
Handling and Safety	No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at www.slingshotbio.com.
Instructions for Use	1. Turn on the flow cytometer and allow it to warm up 30 minutes prior to acquisition of samples and controls. 2. Remove SpectraComp vial from the box. 3. Vortex the vial on high for 2 - 3 seconds to resuspend hydrogel beads. 4. Unscrew the cap on the vial. 5. Add 1 drop of the SpectraComp hydrogels into the bottom of the test tube or well of a plate for the unstained negative control. (1 drop contains approximately 1 x10^5 hydrogels). 6. Add 1 drop of the SpectraComp hydrogels into the bottom of the test tube or well of a plate for each fluorophore you will have in the experiment. 7. Use the same treatment of SpectraComp as you would with cells (i.e. if you are permeabilizing and fixing your cells, you should treat the SpectraComp exactly the same). 8. Add your pre-titrated antibody to the mixture and vortex. Note: It is recommended to pre-determine the appropriate titer of the antibody that works best for the application. 9. Incubate at room temperature for 15 - 30 minutes, protected from light. 10. Add 2 ml of 1X PBS containing 1% BSA (Bovine Serum Albumin) to the tube. Note: Staining buffer containing BSA or FBS (Fetal Bovine Serum) can also be used for washing. 11. Centrifuge the tube for 5 minutes at 600 g and immediately aspirate the supernatant to minimize the hydrogel loss, being careful not to disturb the bead pellet. Note: For best

	signal to noise results, use a vacuum aspirator and aspirate off the supernatant as much as possible. Alternatively, perform two washes by repeating steps 5 and 6 leaving approximately 50µl of supernatant in the tube each time. 12. Resuspend the hydrogel pellet in 1X PBS at preferred volume. Note: Protect the samples from light and analyze the samples as soon as possible. 13. Set the flow cytometer acquisition speed to low. 14. View and acquire the SpectraComp hydrogels on Forward and Side Scatter parameters (FSC-A and SSC-A) using the **same** instrument settings used for actual cells. 15. On the acquisition software, create a gate on the hydrogel population for the negative sample along the forward and side scatter axes. (See Figure 1A.) [image of the bead population here], Then create a gate on the negative histogram (Figure 1B). Create a gate on the hydrogel population for the positive sample (Figure 1C). Then create a gate on the positive histogram for the fluorochrome of the sample (Figure 1D.) Note: It is recommended to use the unstained SpectraComp sample as the negative for each fluorophore that SpectraComp was used.
Storage	SpectraComp should be stored at 2-8°C once the product is received.
Expiration	One year from the date of manufacturing
QC Data	SpectraComp Figure 1 (A, B, C, D)  Figure 1. (A) Gate the SpectraComp population from the negative sample. (B) Place a gate on the negative histogram for the fluorophore of interest from the negative sample. (C) Gate the SpectraComp population from a positive single-stain control sample. (D) Place a gate on the positive histogram for the fluorophore of interest from the positive single-stain control sample.