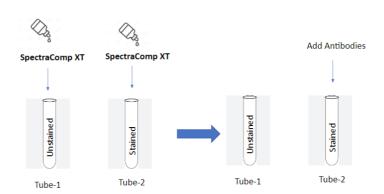
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1. Technical Data Sheet

Summary	SpectraComp [®] XT compensation and unmixing controls are state-of-the-art cell mimics that capture multiple antibody host species (mouse anti-human, mouse, rat, hamster, rabbit and human), and mimic the fluorescence spectra of stained cells.
Application	SpectraComp [®] XT are intended as compensation and unmixing controls to match the single stained performance of real cells. Staining the capture cell mimics yields a positive fluorescence histogram that will aid in resolving the performance of the fluorophore; it will also serve as the basis for the 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 [®] XT are cell mimics that are suspended in aqueous solution and are packaged in a convenient dropper bottle. Each drop contains approximately 1 x10 ⁵ cell mimics.
Handling and Safety	No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at www.slingshotbio.com.
Storage	SpectraComp [®] XT should be stored at 2-8°C once the product is received.
Expiration	One year from the date of manufacturing.
Instructions for Use	 Turn on the flow cytometer and allow it to warm up 30 minutes prior to acquisition of samples and controls. Remove SpectraComp® XT vial from the box. Vortex the vial on high for 2 - 3 seconds to resuspend cell mimics. Unscrew the cap on the vial. Add 1 drop of the SpectraComp® XT cell mimics into the bottom of a test tube or well of a plate for each fluorophore you will have in the experiment. Note: If a true negative is desired, then an unstained SpectraComp® XT sample can be acquired and then applied as a universal negative in your compensation/unmixing matrix. For a true negative, add 1 drop of SpectraComp® XT cell mimics into the bottom of a separate test tube or well of a plate for the unstained negative control. DO NOT add antibody to the unstained tube. See the

illustration below.



- 6. Use the same treatment of SpectraComp $^{\mathbb{R}}$ XT as you would with cells (i.e. if you are permeabilizing and fixing your cells, you should treat the SpectraComp $^{\mathbb{R}}$ XT exactly the same).
- 7. Add your pre-titrated antibody to the $SpectraComp^{\circledR}$ XT cell mimics and vortex. Note: It is recommended to pre-determine the appropriate titer of the antibody that works best for the application.
- 8. Incubate for 15 30 minutes, protected from light.
- 9. Add 2 ml of 1X PBS containing 0.2% BSA (Bovine Serum Albumin) to the tube. Note: Staining buffer containing BSA or FBS (Fetal Bovine Serum) can also be used for washing.
- 10. Centrifuge the tube for 5 minutes at 600 g. Immediately aspirate the supernatant to minimize the cell mimic 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.
- 11. Resuspend the cell mimic pellet in 1X PBS at 200uL or preferred volume. Note: Protect the samples from light and analyze the samples as soon as possible.
- 12. View and acquire the SpectraComp[®] XT cell mimics on Forward and Side Scatter parameters (FSC-A and SSC-A) using the **same** instrument settings used for actual cells.
- 13. For each single stained sample, create a gate on the cell mimic population along the forward and side scatter axes. Then view the cell mimic population in a histogram displaying the proper fluorescence channel for each fluorochrome used. Create one gate on the positive peak and one gate on the negative peak.

 Note: If utilizing an unstained negative SpectraComp® XT sample, make sure to apply the unstained sample as a universal negative in your compensation/unmixing matrix.

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