

# 1. Technical Data Sheet

<b>Summary</b>	HyParComp™ 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.
<b>Application</b>	HyParComp™ are intended as compensation controls to match the single staining performance of real cells. Staining the hydrogels 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.  <b>For Research Use Only. Not for use in diagnostic or therapeutic procedures.</b>
<b>Materials</b>	HyParComp™ are hydrogels that are suspended in aqueous solution and are packaged in a convenient dropper bottle. Each drop contains approximately $1 \times 10^5$ beads.
<b>Handling and Safety</b>	No special handling or safety precautions are necessary. See the Safety Data Sheet (SDS) at <a href="http://www.slingshotbio.com">www.slingshotbio.com</a> .

<p><b>Instructions for Use</b></p>	<ol style="list-style-type: none"> <li>1. Turn on the flow cytometer and allow it to warm up 30 minutes prior to acquisition of samples and controls.</li> <li>2. Remove the HyParComp vials from the box.</li> <li>3. Vortex the vials on high for 2 - 3 seconds to resuspend hydrogel beads.</li> <li>4. Unscrew the caps on the vials.</li> <li>5. Add 1 drop of the negative hydrogels into the bottom of the test tube or well of a plate for the unstained negative control. (1 drop contains approximately <math>1 \times 10^5</math> hydrogels).</li> <li>6. Add 1 drop of the positive capture hydrogels into the bottom of the test tube or well of a plate for each fluorophore you will have in the experiment. (1 drop contains approximately <math>1 \times 10^5</math> hydrogels).</li> <li>7. 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. <b>DO NOT add antibody to the unstained tube.</b></li> <li>8. Use the same treatment of HyParComp as you would with cells (i.e. if you are permeabilizing and fixing your cells, you should treat the HyParComp exactly the same)</li> <li>9. Incubate at room temperature for 15 - 30 minutes, <b>protected from light.</b></li> <li>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.</li> <li>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 hydrogel pellet.</li> <li>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.</li> <li>13. Set the flow cytometer acquisition speed to low.</li> <li>14. View and acquire the HyParComp hydrogels on Forward and Side Scatter parameters (FSC-A and SSC-A) using the <b>same</b> instrument settings used for actual cells.</li> <li>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 HyParComp sample as the negative for each fluorophore that HyParComp was used.</li> </ol>
<p><b>Storage</b></p>	<p>HyParComp™ should be stored at 2 - 8 °C once the product is received.</p>
<p><b>Expiration</b></p>	<p>One year from the date of manufacturing</p>

QC Data

HyParComp Figure 1 (A, B, C, D)

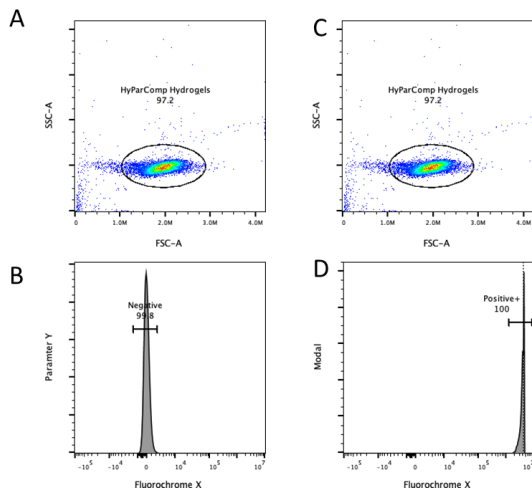


Figure 1. (A) Gate the HyParComp population from the negative sample. (B) Place a gate on the negative histogram for the fluorophore of interest from the negative sample. (C) Gate the HyParComp population from a positive single-stain control sample. (D) Place a gate on the positive histogram for the fluorophore of interest from the positive single-stain control sample.