1. Technical Data Sheet

Summary	HyParComp™ Multipeak compensation and unmixing controls are state-of-the-art hydrogels that capture multiple antibody host species (mouse anti-human, mouse, rat, hamster, and rabbit), and mimic the fluorescence spectra of stained cells.
Application	HyParComp™ Multipeak are intended as compensation and unmixing controls to match the single stained performance of real cells. Staining the hyrodgels yields a positive fluorescence histogram with two positive peaks 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. Note: HyParComp™ Multipeak performance has been verified and validated on analytical flow cytometers and not on cell sorters. **For Research Use Only. Not for use in diagnostic or therapeutic procedures.**
Materials	HyParComp™ Multipeak are hydrogels that are suspended in aqueous solution and are packaged in a convenient dropper bottle. Each drop contains approximately 1 x 10^5 hydrogels.
Handling and Safety	No special handling or safety precautions are necessary. See the Safety Data Sheet (SDS) at www.slingshotbio.com.
Storage	HyParComp™ Multipeak should be stored at 2 - 8 °C once the product is received.
Expiration	18 months from the date of manufacturing.
Instructions for Use	1. Unpack and vortex both vials on high for 2 - 3 seconds to resuspend cell mimics. 2. Add 1 drop of the HyParComp™ Multipeak Negative into the bottom of a test tube or well of a plate for the unstained negative control. (1 drop contains approximately 1 x10^5 hydrogels). 3. Add 1 drop of the HyParComp™ Multipeak Positive into the bottom of a separate test tube or well of a plate for each fluorophore you will have in the experiment. (1 drop contains approximately 1 x10^5 hydrogels). 4. Add your pre-titrated antibody directly to the solution at the bottom of the tube or well and vortex. **Note**: It is recommended to pre-determine the appropriate titer of the antibody that works best for the application. **DO NOT add antibody to the unstained tube**. 5. Incubate for 15 - 30 minutes, **protected from light**.

Note: Hydrogels should generally be treated the same way as cells to be analyzed, i.e. all fixation and permeabilization steps used on cells should be applied to the hydrogels. **However, do not add Brilliant Violet Staining Buffer or SuperBright Staining buffer to hydrogels as it raises the background in the violet region.**

6. Wash by adding 1X PBS to the tube. We recommend using 2 mL to ensure hydrogels are adequately washed.

   Note: Staining buffer containing BSA or FBS (Fetal Bovine Serum) can also be used for washing.

7. Centrifuge the tube for 5 minutes at 600 g. Immediately aspirate the supernatant to minimize the hydrogel loss, being careful not to disturb the hydrogel pellet.
8. Resuspend the hydrogel pellet in 1X PBS or stain buffer at preferred volume.

   Note: Protect the samples from light and analyze the samples as soon as possible.

9. View and acquire HyParComp™ Multipeak using the same instrument settings as leukocytes.
10. Gate on the positive peak that is more suitable for your compensation/unmixing application.
11. It is recommended to designate the unstained HyParComp™ Multipeak Negative as an external negative for compensation/unmixing workflows requiring such.

QC Data

Single Stained Positive profile