1. Technical Data Sheet

Summary	ViaComp® Ultra™ XT - Amine compensation/unmixing controls are state-of-the-art cell mimics that bind amine-reactive viability dyes and capture multiple antibody host species (mouse, rat, hamster, rabbit, human), providing a fluorescence spectra for the dye or antibody they are stained with.
Application	ViaComp® Ultra™ XT - Amine is intended to be used as compensation/unmixing and assay controls to match the staining performance of real cells. Staining the cell mimics with amine-reactive viability dyes or fluorophore-conjugated antibodies yields a positive peak and a negative peak for use as single-stained controls or as a gating control identifying live and dead cell populations. Note: ViaComp® Ultra™ XT - Amine is not compatible with DNA-intercalating dyes (e.g. 7-AAD, PI, DAPI, etc.) Note: ViaComp® Ultra™ XT - Amine 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	ViaComp® Ultra™ XT - Amine are cell mimics that are suspended in aqueous solution and are packaged in a convenient dropper bottle. Each drop contains approximately 6.6×10^4 cell mimics.
Handling and Safety	No special handling or safety precautions are necessary. See SDS at www.slingshotbio.com.
Storage	ViaComp® Ultra™ XT - Amine should be stored at 2-8°C once the product is received.
Expiration	18 months from the date of manufacturing.

Instructions for Use	**Staining with amine-reactive viability dyes** 1. Remove ViaComp® Ultra™ XT - Amine from 2–8°C storage. 2. Vortex bottle on high for 2-3 seconds at least 5 times to ensure the cell mimics are fully resuspended. 3. Add 1 drop of cell mimics into the tube or plate well. Ensure the drop goes to the bottom of the tube/well. 4. Prepare amine-reactive dye dilution in 1X PBS, or as directed by the vendor. *Note: It is recommended to pre-determine the appropriate titer of the viability dye that works best for your application.* 5. Add the diluted dye to the cell mimics. Vortex mixture on high for 2 seconds to mix the tube thoroughly or pipette to mix thoroughly on the plate. 6. Incubate at room temperature for 20-30 min, protected from light. *Note: Refer to the specific vendor's instructions for the incubation time if different.* 7. Centrifuge the tube/plate for 5-6 minutes at 500 x g. Remove excess supernatant without disturbing the pellet. 8. Wash by resuspending the bead pellet in 2 mL Stain Buffer containing 0.2-1% BSA (Bovine Serum Albumin) or equivalent and then vortex or pipette to resuspend the pellet. *Note: If using a plate, the recommended wash volume is 200* µL. 9. Repeat steps 7 and 8 to wash particles thoroughly. Resuspend the cell mimics in the desired volume of Stain Buffer or 1X PBS (~200 µL). 10. Analyze on a flow cytometer using the same FSC and SSC settings as leukocytes. *Note: For best resolution, use a low flow rate.* **Staining with fluorophore-conjugated antibodies** 1. Remove ViaComp® Ultra™ XT - Amine from 2–8°C storage. 2. Vortex bottle on high for 2-3 seconds at least 5 times to ensure the cell mimics are fully resuspended. 3. Add 1 drop of cell mimics into the tube or plate well for each single color control. Ensure the drop goes to the bottom of the tube/well.

Note: ViaComp® Ultra™ XT - Amine is formulated to provide an internal negative population that is not designed to bind antibodies. You have the option to use this as a negative population for compensation/unmixing workflows, however it is recommended to prepare a separate unstained ViaComp® Ultra™ XT - Amine tube/well for a universal negative.

4. Add the appropriate volume of antibody to the cell mimics. Vortex mixture on high for 2 seconds to mix the tube thoroughly or pipette to mix thoroughly on the plate.

   Note: It is recommended to pre-determine the appropriate titer of the antibody that works best for your application.

6. Incubate at room temperature for 20-30 minutes, protected from light.

   Note: Use the same treatment of ViaComp® Ultra™ XT - Amine as you would with cells (i.e. if you are permeabilizing and fixing your cells, you should treat ViaComp® Ultra™ XT - Amine exactly the same).

8. Add 2 mL of Stain Buffer containing 0.2-1% BSA to the tube.

   Note: If staining using a plate it is recommended to add 100 µL for the initial wash and 200 µL for any subsequent washes.

   Note: Stain Buffer containing BSA (Bovine Serum Albumin) or FBS (Fetal Bovine Serum) can be used for washing.

9. Centrifuge the tube for 5-6 minutes at 500 x g. Remove excess supernatant without disturbing the pellet.
10. For maximal signal to noise ratio, repeat steps 8 and 9 at least once for a total of 2 washes or more.
11. Resuspend the cell mimics in desired volume of Stain Buffer or 1X PBS (~200 µL).
12. Analyze on a flow cytometer using the same FSC and SSC settings as leukocytes.

    Note: Protect the samples from light and analyze the samples as soon as possible. For best resolution, use a low flow rate.

QC Data

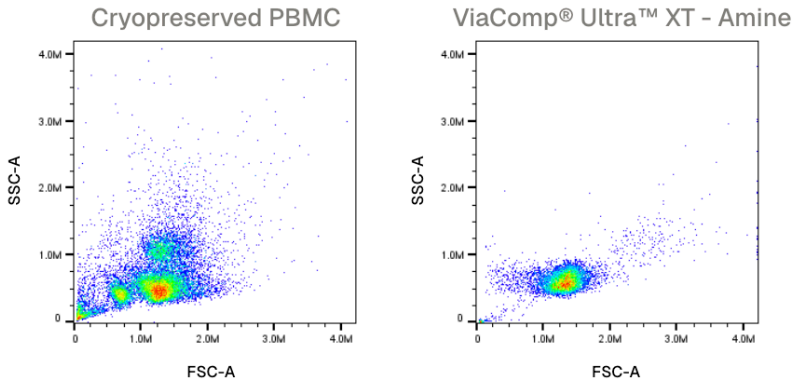

Figure 1. Scatter profiles of (left) cryopreserved PBMC vs. (right) ViaComp® Ultra™ XT - Amine.

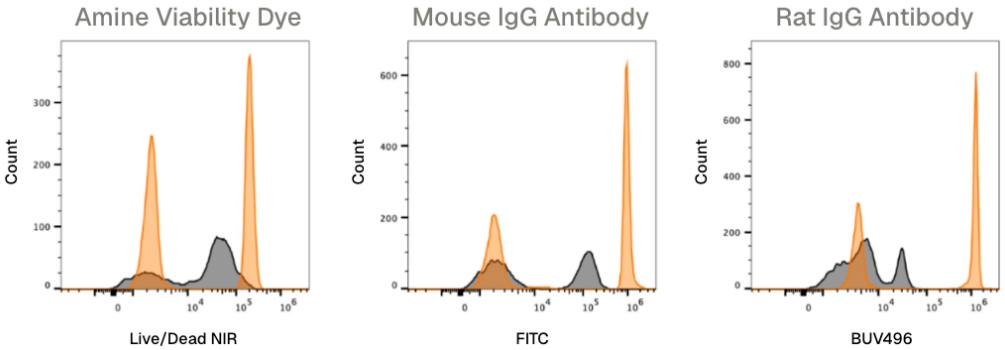

Figure 2. ViaComp® Ultra™ XT - Amine (orange) vs. cryopreserved PBMC (grey), stained with (left) LIVE/DEAD Near IR, (middle) Mouse IgG FITC, and (right) Rat IgG BUV496.

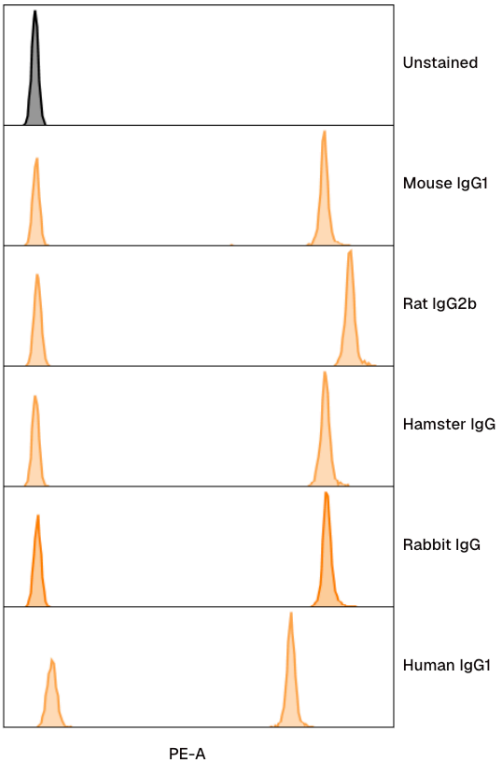

Figure 3. ViaComp® Ultra™ XT - Amine unstained and stained with PE-conjugated antibodies from different host species.

Technical Support	For technical support regarding this product please contact: support@slingshotbio.com