

1. Technical Data Sheet

Summary	TruCytes™ CD34+ Stem Cell Gating Control is a lyophilized cell mimic control that features CD34, CD45, and viability (DNA) biomarkers with scatter coordinates that closely mimic lymphocyte populations. This product is a high density CD34+ control with CD34+ frequencies comparable to biological samples such as human cord blood, bone marrow, and mobilized peripheral blood cells (~90% CD34+ as a percentage of CD45+ cells). It is intended to provide positive signal detection for CD45 and positive and negative signal detection for CD34 and viability.																		
Application	The product is intended for use as a flow cytometry gating control and antibody qualification for the detection of CD34 and CD45 with the option for viability staining with DNA-binding dyes. This product is intended to provide positive signals for specified biomarkers and their antibodies listed in the table below: <table border="1"> <thead> <tr> <th>Biomarker</th><th colspan="3">Clone/Reagent</th></tr> </thead> <tbody> <tr> <td>CD45</td><td>2D1</td><td>HI-30</td><td>J33</td></tr> <tr> <td>CD34</td><td>8G12</td><td>561</td><td>4H11</td></tr> <tr> <td>Viability</td><td colspan="3">7-AAD (DNA-based staining)</td></tr> </tbody> </table> Note: Antibody clones and viability reagents not listed above need to be tested independently to determine compatibility. This product is not compatible with viability dyes that stain for amines. For Research Use Only. Not for use in diagnostic or therapeutic procedures.			Biomarker	Clone/Reagent			CD45	2D1	HI-30	J33	CD34	8G12	561	4H11	Viability	7-AAD (DNA-based staining)		
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CD45	2D1	HI-30	J33																
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Materials	This product is lyophilized for stability and ease of use. Each vial contains 2.5×10^5 cell mimics.																		
Handling and Safety	No special handling or safety precautions are necessary.																		
Storage	Store lyophilized products at -20 °C upon receipt.																		
Expiration	12 months from the date of manufacturing when stored at -20 °C. Use the entire vial immediately upon reconstitution of lyophilized product.																		
Instructions for Use	1. Remove the vial of TruCytes™ CD34+ Stem Cell Gating Control from the -20 °C and let it sit at room temperature for 15 minutes.																		

(Sample Preparation)

2. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial.
3. Add 250 µL of staining buffer to the vial.
4. Note: Avoid contacting or disturbing the pellet until it has been hydrated (~1 minute).
5. Gently pipette up and down ten times to resuspend the cell mimics. Avoid introducing bubbles. Transfer the contents to a 5mL FACS tube.
6. Add 1000 µL of flow staining buffer to the original vial, mix by gentle pipetting, and transfer the remaining of the mimics to the FACS tube prepared in the previous step for a final volume of 1250 µL.
7. Centrifuge at 600 x g for 5 minutes.
8. Decant the supernatant being careful not to disturb the cell pellet.
9. The cell mimics are ready for staining.

Product can be stained with or without a DNA-binding viability dye. Follow Protocol A when staining with a viability dye. If it is preferred not to use a viability dye in the staining, follow Protocol B.

Staining Protocol A Instructions for Use (with DNA-binding viability dye)

1. Prepare your preferred staining antibody cocktail in flow staining buffer and add to washed cell mimics.
2. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 15 +/- 3 min.
3. Add 1000 µL of flow staining buffer.
4. Centrifuge at 600 x g for 5 minutes.
5. Decant the supernatant being careful not to disturb the cell pellet.
6. Add 100 µL of flow staining buffer.
7. Add desired volume of 7-AAD staining dye.
8. Incubate in the dark for 10 minutes.
9. Add 400 µL of flow staining buffer and mix thoroughly by pipette mixing.

The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as your biological samples. For best results, we recommend acquiring your sample immediately using low or medium flow rate.

Representative QC Data with Staining Protocol A

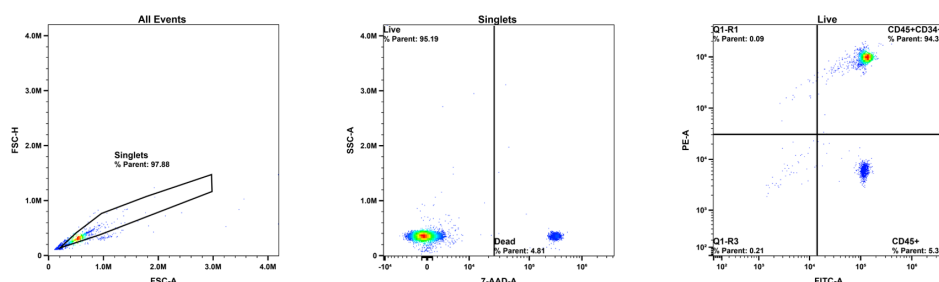


Figure 1. Representative dot plots for viability and CD34 gating strategy with TruCytes™ CD34+ Stem Cell Gating Control. The product was stained using anti-CD34 PE, anti-CD45 FITC and 7-AAD viability dye.

Staining Protocol B Instructions for Use (without DNA-binding viability dye)

1. Prepare your preferred staining antibody cocktail in flow staining buffer and add to washed cell mimics.
2. Incubate the mixture at room temperature in the dark as per your specific antibody panel requirements. Generally, we recommend 15 +/- 3 min.
3. Add 1000 μ L of flow staining buffer.
4. Centrifuge at 600 x g for 5 minutes.
5. Decant the supernatant being careful not to disturb the cell pellet.
6. Add 400 μ L of flow staining buffer and mix thoroughly by pipette mixing.

The sample is ready for acquisition - acquire cell mimics using the same flow cytometer instrument settings as your biological samples. For best results, we recommend acquiring your sample immediately using low or medium flow rate.

Representative QC Data with Staining Protocol B

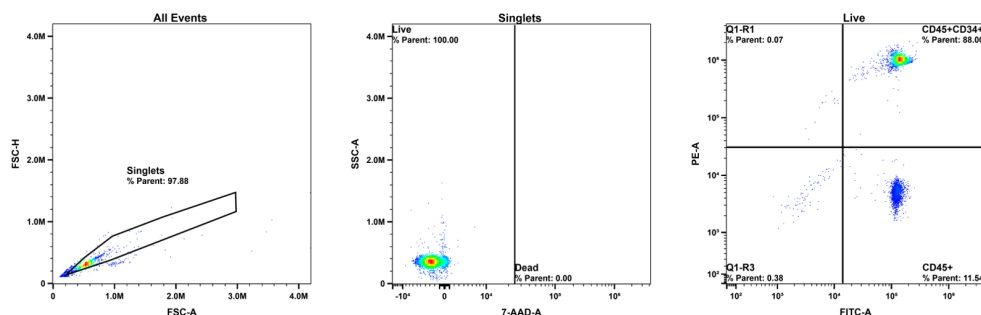


Figure 2. Representative dot plots for viability and CD34 gating strategy with TruCytes™ CD34+ Stem Cell Gating Control. The product was stained using anti-CD34 PE and anti-CD45 FITC. No viability dye was added during the analysis.

**Technical
Support**

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