1. Technical Data Sheet

Summary	TruCytes™ CD19 Quantitative Antigen Density controls are lyophilized cell mimics that feature the CD19 biomarker with scatter properties resembling human lymphocytes.
Application	TruCytes™ CD19 Quantitative Antigen Density controls provide a quantitative amount of CD19 including a negative and three positive peaks. Each positive level varies in the number of CD19 protein, giving each population a distinct fluorescence intensity and antigen density level.
Materials	TruCytes™ CD19 Quantitative Antigen Density controls are lyophilized cell mimics in a glass vial. Each vial contains approximately 2.5 x 10^5 cell mimics.
Handling and Safety	No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at www.slingshotbio.com.
Storage	TruCytes™ CD19 Quantitative Antigen Density product should be stored at -20 °C upon receipt and used immediately once reconstituted.
Expiration	Twelve months from the date of manufacturing.
Instructions for Use	**Note:** Staining and acquisition needs to be performed on the same day as test samples for accurate quantification. 1. **<u>Staining Instructions</u>** 1.1. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial. 1.2. Add 1000 µL flow staining buffer (0.2% BSA, PBS, 0.09% sodium azide) to each lyophilized vial. Swirl and transfer the contents of the vial to its own individual FACS tube. 1.3. Centrifuge at 500 x g for 5 minutes. 1.4. Remove 900 µL supernatant to leave 100 µL in the FACS tube. 1.5. Add 1000 µL flow staining buffer. Repeat steps 3 and 4 resulting in 200 µL being left in each FACS tube. 1.6. Add 1µg of antibody in 100 µL of flow staining buffer (clone: HIB-19, fluorophore: PE; the manufacturer's recommended

volume is typically 2-5 µL) to the same tube. Vortex for 3 seconds.

1.7. Incubate at RT in the dark for 30 min.

1.8. Wash by adding 1000 µL of staining buffer. Mix well, then centrifuge at 500 x g for 5 minutes. Remove the supernatant to leave ~100 µL in the FACS tube.

1.9. Repeat the previous wash step once more. Add desired volume of staining buffer to the tube/well after the final wash.
Note: For accurate quantification, a total of two washes are recommended.

1.10. Acquire each FACS tube using the same FSC and SSC settings as leukocytes.

2. **Data Analysis Directions Note:** A downloadable calculator is available to generate the required formula and calculate antigen density. Please access the tool here: Antigen Density Calculator (https://shop.slingshotbio.com/products/ctx-trucytes-cd19) .
The methods for manual analysis are listed below.

2.1. **Standard Curve Determination**

2.1.1. Properly gate the population for CD19 Quantitative Antigen Density Kit as in Figure 2 below in a plot of SSC-A versus FSC-A.

2.1.2. In the channel for your anti-CD19 fluorophore, gate on each subpopulation of CD19 Quantitative Antigen Density Kit to obtain three MFIs for each of the negative, low, medium, and high peaks. From the high, medium, and low peak MFIs, subtract the negative peak. For example, see below:

	Negative Peak	Low Peak	Medium Peak	High Peak
Obtained MFI	472	53032	246123	831095
Background subtracted MFI	0	52560	245651	830623

Table 1. Demonstration of subtracting blank MFI from quantitative peaks.

2.1.3. Plot the lot-specific antigen densities against blank-subtracted MFIs on a linear plot. This will be your blank-subtracted standard curve for your data analysis below. Use a linear regression for the curve-of-best-fit.

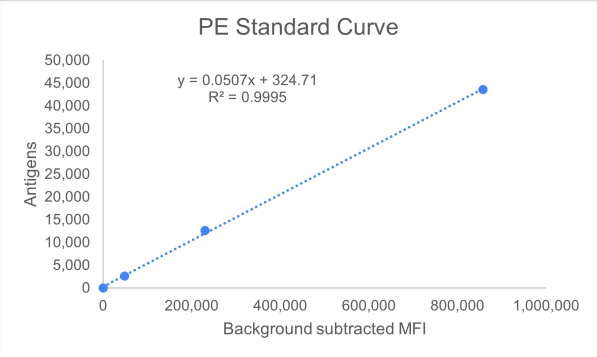


Figure 1. Standard curve of antigens per cell versus blank-subtracted MFI.

3. **Unknown sample analysis**

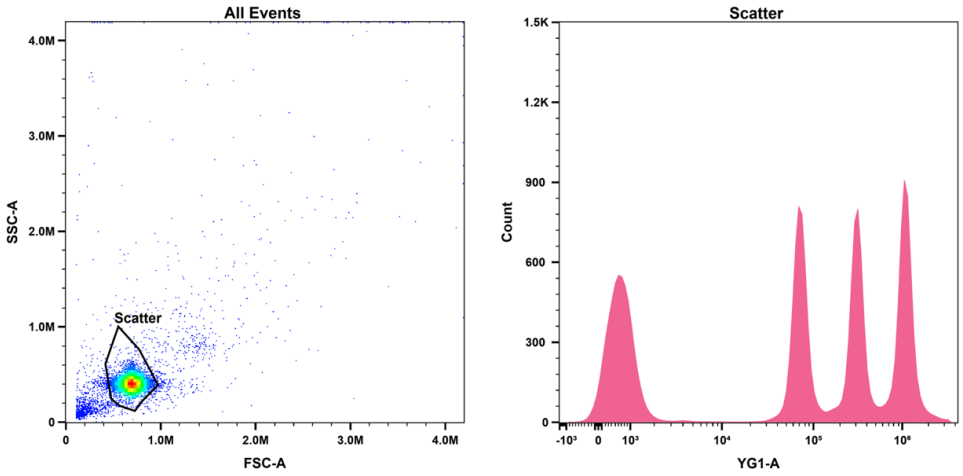
3.1. After employing your assay-specific gating strategy, gate on the target population to determine the MFI of the target cell population within the channel corresponding to the anti-CD19 antibody. Do the same analysis for all isotype-stained controls.

3.2. To generate data points to analyze on your standard curve, subtract your isotype control MFI from each sample's MFI. Each cell type requires their own isotype control staining as background staining varies between cell types. These are now your blank-subtracted MFIs.

3.3. Use your regression equation above to analyze each sample for antigen density.

3.4. Please refer to https://slingshotbio.com/resources for the COA containing antigen density numbers to perform linear regression.

QC Data

	 Figure 2. Left: Scatter plot of lymphocyte mimics. Right: Representative flow cytometry analysis of stained TruCytes™ CD19 Quantitative Antigen Density controls with PE-HIB19.
Technical Support	For technical support regarding this product please contact: support@slingshotbio.com