1. Technical Data Sheet

Summary	TruCytes™ BCMA Quant controls are lyophilized cell mimics that feature the BCMA biomarker with scatter properties resembling human lymphocytes.
Application	TruCytes™ BCMA Quant controls provide a quantitative amount of BCMA including a negative and three positive peaks. Each positive level varies in the number of BCMA protein, giving each population a distinct fluorescence intensity and antigen density level.
Materials	TruCytes™ 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™ BCMA product should be stored at -20 °C upon receipt and used immediately once reconstituted.
Expiration	Eighteen months from the date of manufacturing.
Instructions for Use	**Note:** Staining and acquisition of TruCytes™ needs to be performed on the same day as test samples for accurate quantification. <u>Staining Instructions</u> 1. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial. 2. Add 1000 µL of flow staining buffer (0.2% BSA, PBS, 0.09% sodium azide) to the vial and transfer the contents to desired container for staining. 3. Centrifuge at 500 x g for 5 minutes and remove the supernatant without disturbing the cell pellet. Remove the supernatant, being careful to not disturb the pellet. 4. Add 1000 µL of flow staining buffer. Centrifuge at 500 x g for 5 minutes and remove the supernatant without disturbing the cell pellet. Remove the supernatant, being careful to not disturb the pellet. 5. Add 100 µL of flow staining buffer to the pellet. 6. Add 1 µg staining antibody (clone: 19F2, fluorophore: PE; the manufacturer's recommended volume is typically 2-5 µL) to the same tube. Vortex for 3 seconds.

7. Incubate at room temperature in the dark for 30 min.
8. Wash by adding 1 mL of flow staining buffer. Mix well, then centrifuge at 500 x g for 5 minutes. Remove the supernatant without disturbing the pellet.
9. Repeat the previous wash step once more.

   Note: **For accurate quantification, a total of two washes are required.**
10. Acquire using the same FSC and SSC settings as leukocytes.

Please note the following considerations when using TruCytes™ BCMA :

- Stain TruCytes™ with the same primary antibody and fluorophore conjugate as the test samples.
- Analyze TruCytes™ BCMA and cells immediately after the final post-stain wash
- TruCytes™ have not been evaluated for use with secondary antibody staining.
- For accurate quantification of cells of interest, isotype staining to determine background staining of the cells of interest is recommended. This is particularly important when you anticipate antigen density below 1000 per cell.
  - If isotype staining is not feasible, the negative peak in the TruCytes™ may be used instead as a rough approximation for background fluorescence. This assumption may impact quantitation when comparing different cell types.
- To obtain similar antigen numbers on cells we recommend using clone 19F2 with fluorophore PE. Other clone/fluorophore combinations have not been validated and must be tested empirically.

1. Data Analysis Directions

   Note: A downloadable calculator is available to generate the required formula and calculate antigen density. Please access the Antigen Density Calculator here: https://shop.slingshotbio.com/products/trucytes%E2%84%A2-quantitative-bcma-cell-mimic.
   The methods for manual analysis are listed below.
   Please refer to the Certificate of Analysis (link above) for antigen density numbers to perform linear regression:

a. Standard curve determination

i. Properly gate the population for TruCytes™ as in Figure 1 below in a plot of SSC-A versus FSC-A.

ii. In the channel for your anti-BMCA fluorophore, gate on each subpopulation of TruCytes™ 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	10607	78373	245482	861000
Blank-subtracted MFI	0	67766	234875	850393

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

iii. 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.

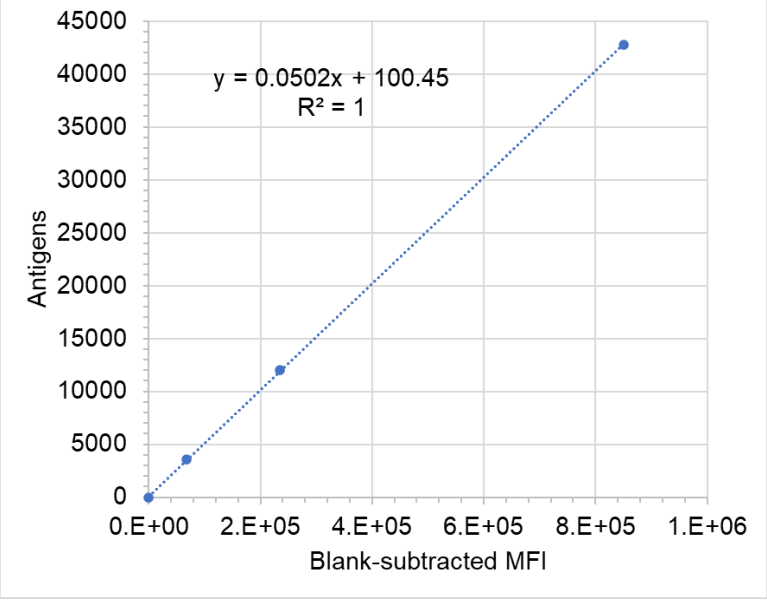

Figure 1. Standard curve of antigens per cell versus blank-subtracted MFI.

iv.

b. Unknown sample analysis

i. 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-BCMA antibody. Do the same analysis for all isotype-stained controls.

ii. 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.

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

Representative Data

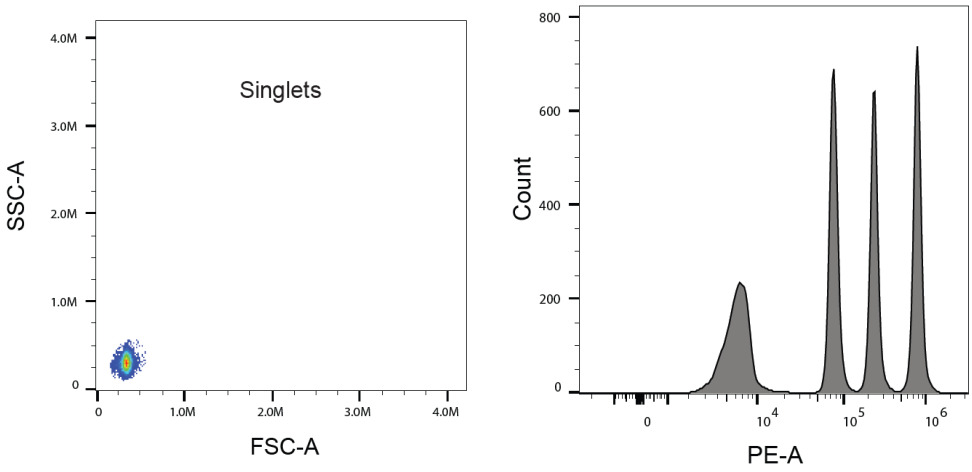

Figure 2. Left: Scatter plot of lymphocyte mimics. Right: Representative flow cytometry analysis of stained TruCytes™ BCMA Quant.

Technical Support

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