Technical Data Sheet

Slingshot Biosciences   TDS-33
CTx TruCytes™ BCMA Quantitative Antigen Density (P/N: NPI-00039) Technical Data Sheet   Version: 1.0

1. Technical Data Sheet

Summary	CTx TruCytes™ BCMA Quantitative Antigen Density controls are lyophilized cell mimics that feature the BCMA biomarker with scatter properties resembling human lymphocytes.
Application	CTx TruCytes™ BCMA Quantitative Antigen Density 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 2.5×10^5 cell mimics.
Handling and Safety	No special handling or safety precautions are necessary. See Safety Data Sheet (SDS) at www.slingshotbio.com.
Storage	CTx TruCytes™ BCMA 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 of CTx TruCytes™ needs to be performed on the same day as test samples for accurate quantification. 1.1. Staining Instructions 1. Tap down the vial to ensure that all cell mimics are collected at the bottom of the vial. 2. Add 250 μL of PBS (see note below) to the vial and transfer the contents to desired container for staining. Add 1mL of PBS to the glass vial and transfer residual beads to the same staining container. **Note**: **Using 1% BSA in PBS will improve yield during reconstitution. MilliQ grade water or higher is recommended as a substitute if PBS is not available.** 3. Centrifuge at 500 x g for 5 minutes or 16000 x g for 30 seconds and remove the supernatant without disturbing the cell pellet. Resuspend the pellet in the desired staining buffer at your desired staining volume. Slingshot Biosciences' scientists use a final staining volume of 100 uL. 4. Add an appropriate amount of your staining antibody and mix well by vortexing.

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

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

6. Wash by adding 1 mL of staining buffer. Mix well, then centrifuge at 500 x g for 5 minutes or 16000 x g for 30 seconds. Remove the supernatant without disturbing the pellet.

   Note: **FACS tubes provide optimal washing due to higher volume capacity.**

7. 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 required.**

8. Acquire using the same FSC and SSC settings as leukocytes.

Please note the following considerations when using CTx TruCytes™ BCMA :

- Stain CTx TruCytes™ with the same primary antibody and fluorophore conjugate as the test samples.

- CTx TruCytes™ have not been evaluated for use with secondary antibody staining.

- For accurate quantification of cells of interest, isotype staining to determine background staining 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 CTx 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 either fluorophore PE or APC. 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 tool here: BCMA Antigen Density Calculator. The methods for manual analysis

are listed below. Please refer to Certificate of Analysis for antigen density numbers to perform linear regression.

a. <u>Standard curve determination</u>

   i. Properly gate the population for CTx 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 CTx 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.
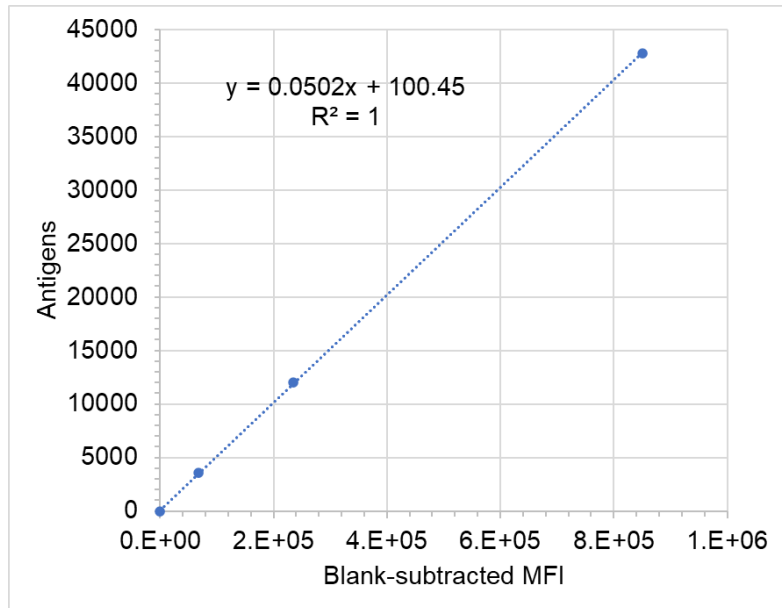
Property of Slingshot Biosciences. **CONFIDENTIAL**. Unauthorized distribution or copying prohibited.
Generated by Caitlin Farquhar at 2023-10-17 11:11:13 (UTC -0600) Uncontrolled copy. Valid for 24 hours only.

Page 3 of 5

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.

QC Data

Property of Slingshot Biosciences. **CONFIDENTIAL**. Unauthorized distribution or copying prohibited.
Generated by Caitlin Farquhar at 2023-10-17 11:11:13 (UTC -0600) Uncontrolled copy. Valid for 24 hours only.
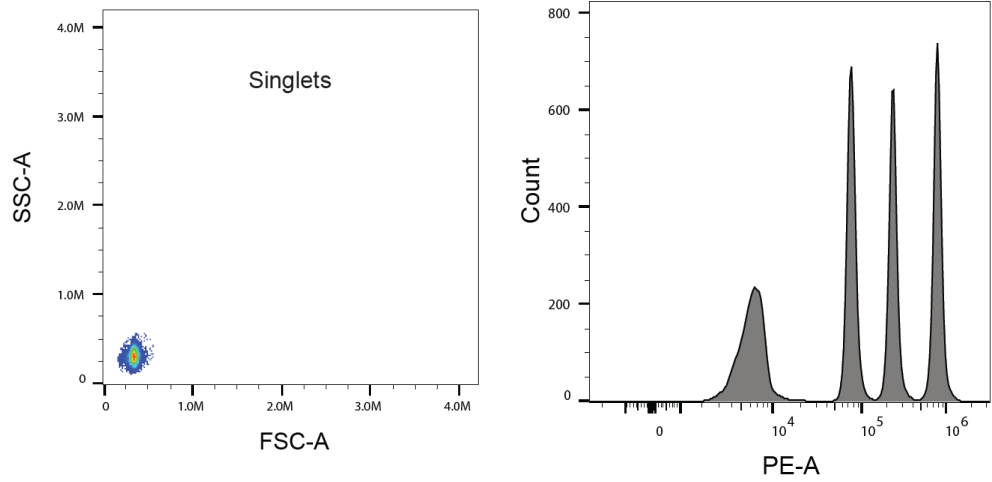
Page 4 of 5

Figure 2. Left: Scatter plot of lymphocyte mimics. Right: Representative flow cytometry analysis of stained CTx TruCytes™ BCMA Quantitative Antigen Density.

Property of Slingshot Biosciences. **CONFIDENTIAL**. Unauthorized distribution or copying prohibited. Generated by Caitlin Farquhar at 2023-10-17 11:11:13 (UTC -0600) Uncontrolled copy. Valid for 24 hours only.

Page 5 of 5