Technical Data Sheet

Slingshot Biosciences   TDS-44
CTx TruCytes™ CD19 Quantitative Antigen Density (P/N: NPI-00036) Technical Data Sheet   Version: 1.0

1. Technical Data Sheet

Summary	CTx TruCytes™ CD19 Quantitative Antigen Density controls are lyophilized cell mimics that feature the CD19 biomarker with scatter properties resembling human lymphocytes.
Application	CTx 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™ 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™ CD19 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 desired staining buffer. 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. Saturating amounts of antibody will allow for accurate quantification.

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 recommended.

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

Please note the following considerations when using CTx TruCytes™ CD19:

- 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.

- Negative quantitation and all references from Slingshot Biosciences for this product was performed with isotype controls to account for background staining fluorescence. 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 HIB19 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: Antigen Density Calculator

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(https://shop.slingshotbio.com/products/ctx-trucytes-cd19) . The methods for manual analysis are listed below.

  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-CD19 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	3539	52390	233795	861000
Blank-subtracted MFI	0	48851	230256	857461

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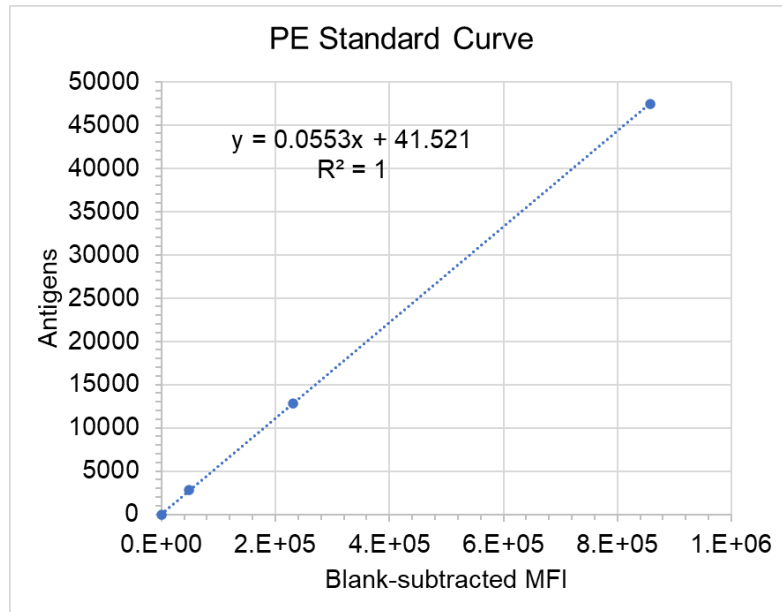
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Figure 1. Standard curve of antigens per cell versus blank-subtracted MFI.

a. <u>Unknown sample analysis</u>

    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.

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

QC Data

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Figure 2. Left: Scatter plot of lymphocyte mimics. Right: Representative flow cytometry analysis of stained CTx TruCytes™ CD19 Quantitative Antigen Density with PE-HIB19.