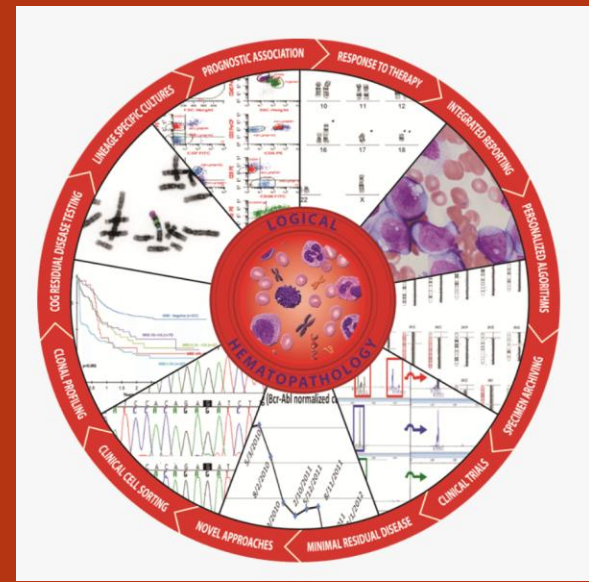




Background

Quality control (QC) of multidimensional flow cytometry frequently depends on donor-derived specimens, which are limited by biological variability and availability, or polystyrene beads that often fail to accurately replicate cell characteristics. These limitations are particularly challenging for rare antigens that are not expressed on normal hematopoietic cells, making it difficult to obtain appropriate biological controls for assessing assay performance, sensitivity, and reproducibility.

Trucytes are lyophilized cell mimics with scatter characteristics (FSC and SSC) that correspond to the optical properties of real cell populations and feature selected biomarkers.

In this study, we investigate the use of Trucytes for QC of HematoLogics diagnostic biomarker or T-cell measurable residual disease assays. For the diagnostic biomarker panel, multiple Trucytes populations were designed to stand alone, while the T-MRD panel, Trucytes will be spiked to a bone marrow specimen.

Methods and Materials

Six custom cell mimic populations were engineered with lymphocyte or monocyte scatter characteristics expressing targets included in the HematoLogics diagnostic biomarker or T-cell measurable residual disease assays (Table 1). The HematoLogics diagnostic biomarker panel assesses potential therapeutic targets, many of which are not expressed on normal hematopoietic cells.

Table 1. Description of cell mimic populations and antigens.

Product description	Target scatter properties	Target biomarkers
Base particles	Lymphocytes	CD45 PerCP, CD38 FITC, CD34 APC, CD3 APC, CD1a FITC
Spike 1	Monocytes	CD371 PE, Mesothelin PE
Spike 2	Monocytes	CD276 PE, TSLPR PE
Spike 3	Monocytes	CD74 PE, FOLR1 PE, CD56 PE
Spike 4	Monocytes	CD70 PE, CSPG4 PE
Spike 5	Monocytes	CLEC2A PE, CD131 PE

Mean fluorescence intensity (MFI) targets were established using Rainbow 8-peak calibration particles.

Performance of cell mimics was evaluated by scatter characteristics, antigen expression, specificity, and compatibility with existing workflows.

A 24-month accelerated stability study of the custom cell mimics was conducted to show that Trucytes remain identifiable at the expected percentages, identity, and MFI over a 24-month equivalent using the Arrhenius equation.

The Arrhenius equation is an expression that shows how the rate constant of a chemical reaction, absolute temperature, and the aging factor are related.

Results

Trucytes display specific staining

Trucytes exhibited low autofluorescence, minimal nonspecific staining, and clear separation from FMO controls (**Figure 1**).

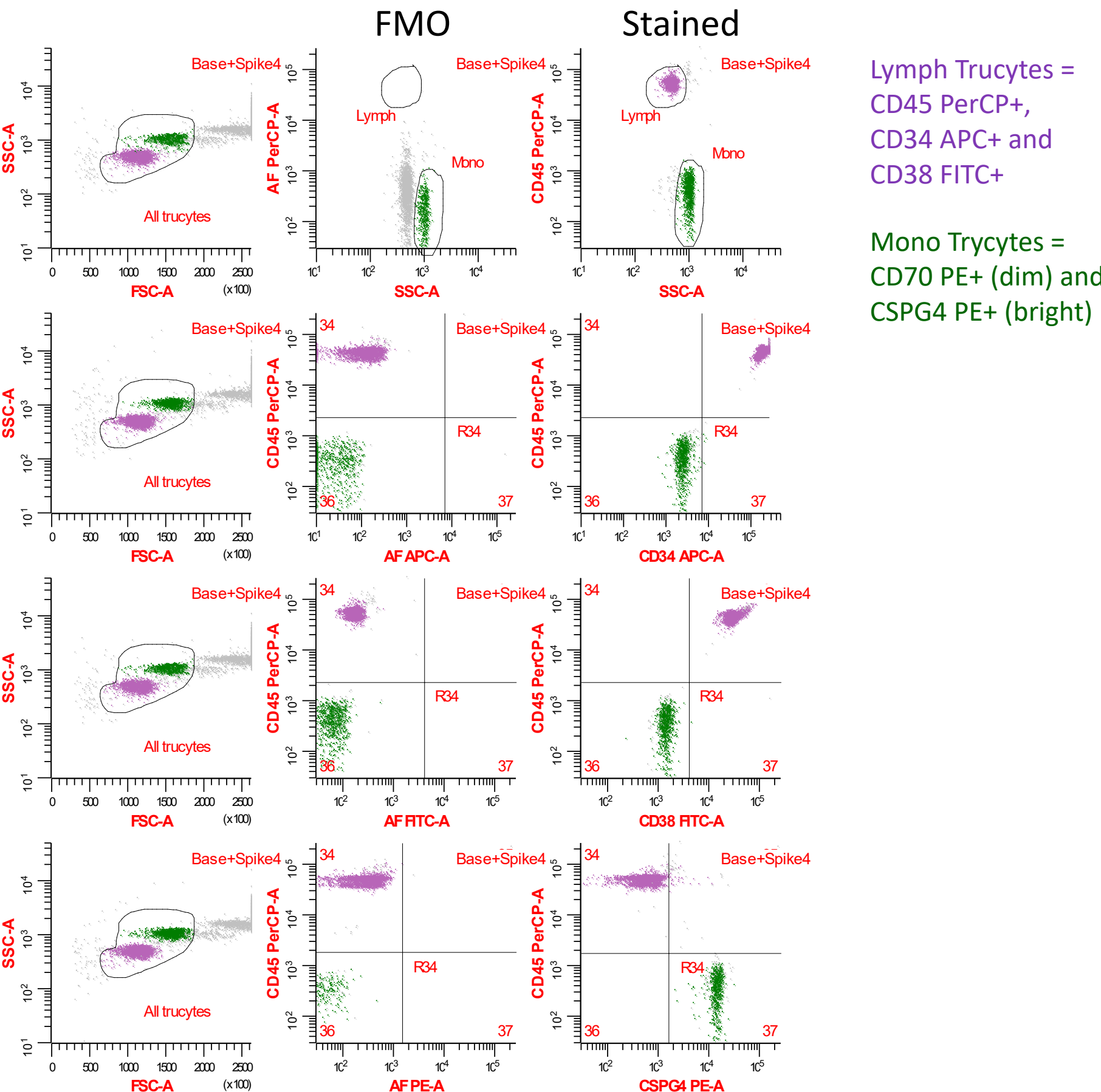


Figure 1. Representative flow cytometric plots showing examination of scatter properties, autofluorescence and non-specific signal of Trucytes.

Trucytes expressed all targets within predefined MFI ranges

Cell mimics expressed all biomarkers at target MFI. Each spike particle expressed two PE-conjugated antigens which could be discriminated based on staining intensity (**Figure 2**). CD34 APC expression was at the upper limit of the target range, therefor APC targets were successfully revised (data not shown).

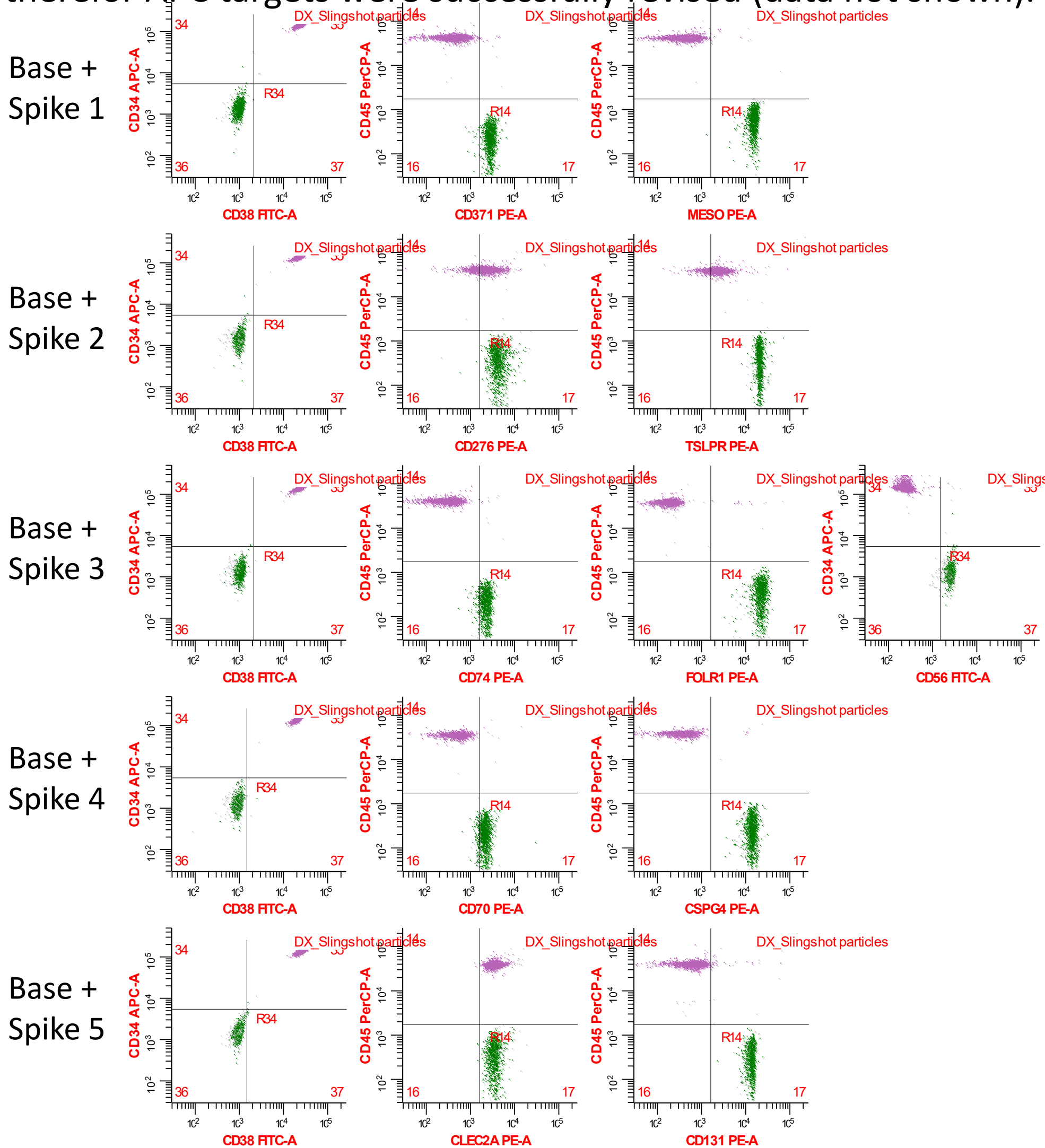


Figure 2. Representative flow cytometric plots showing Trucytes staining performance for target biomarkers.

Trucytes are distinguishable from biological cells

Trucytes remain identifiable and discriminate from bone marrow cells when spiked-in. Furthermore, Trucytes retained good staining quality when stained together (**Figure 3**).

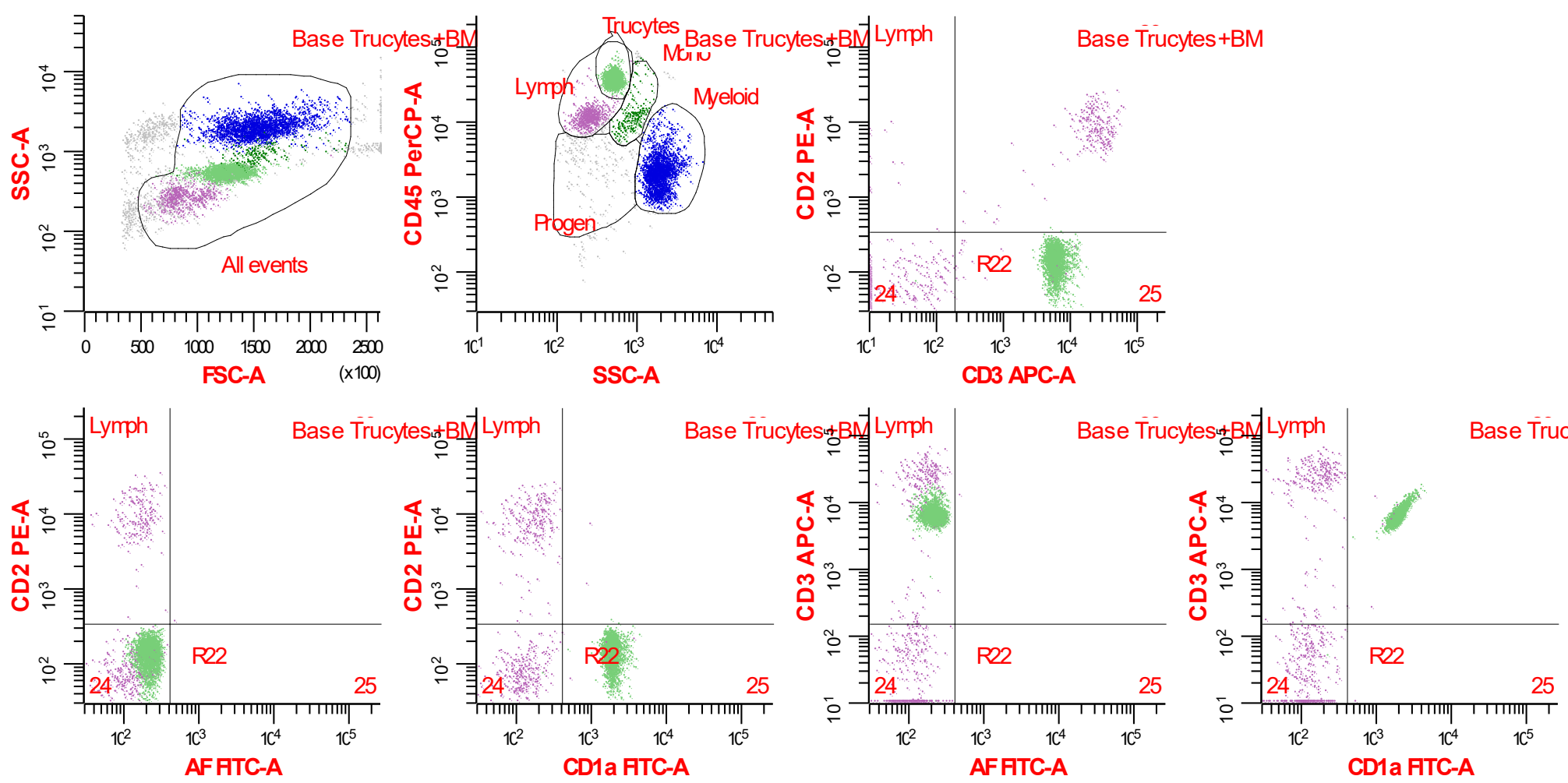


Figure 3. Base particles mixed with human bone marrow and stained with MRD tube (CD1a FITC/CD2 PE/CD45 PerCP/CD3 APC).

Trucytes are stable for at least two-years

A 2-year stability study demonstrated that all Trucytes have acceptable variability, with %CV values remaining below 10% across all markers (**Figure 4**).

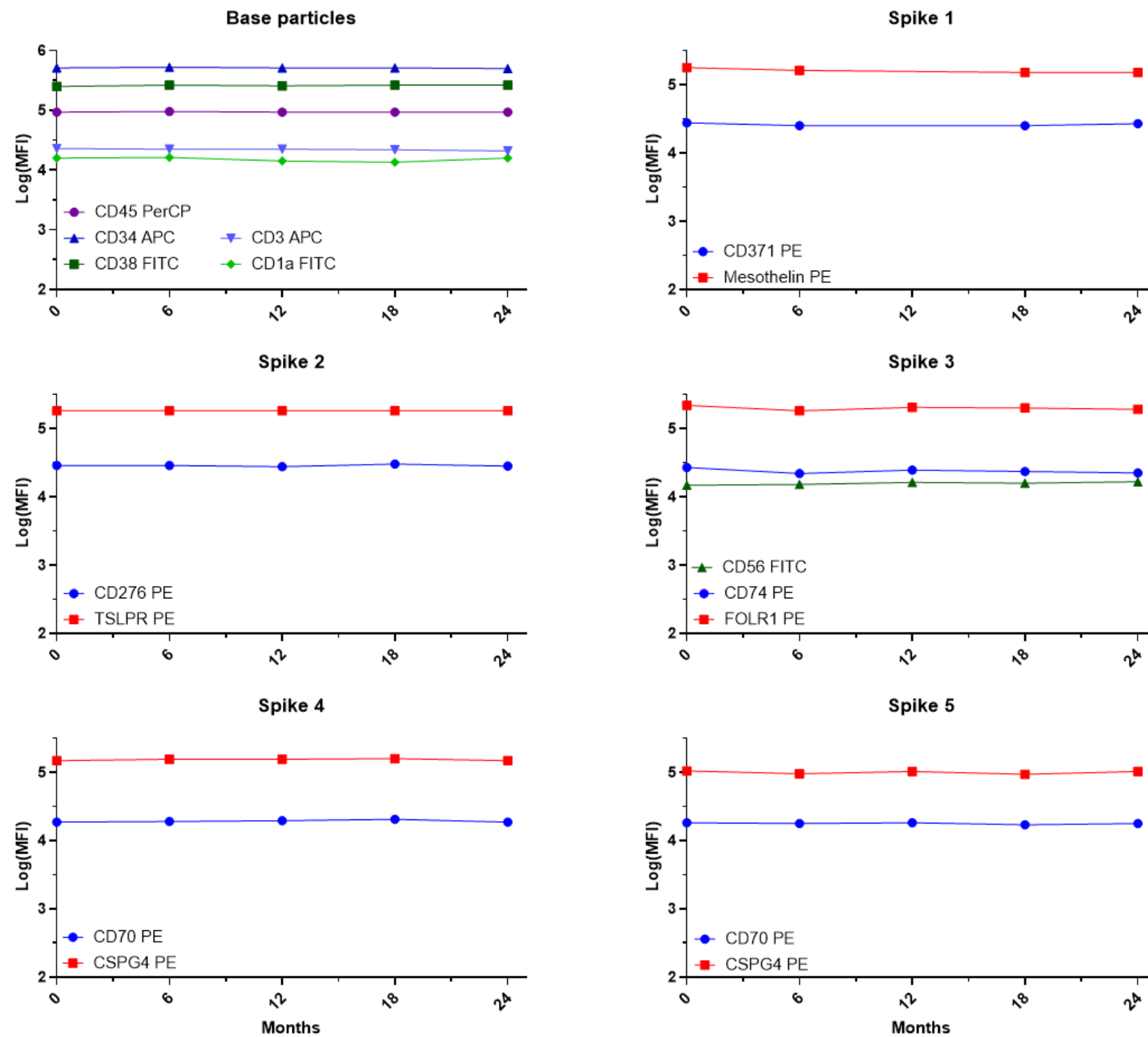


Figure 4. MFI of Trucytes over a simulated 24-months stability study.

Conclusions

- Custom Trucytes cell mimics successfully expressed the target biomarkers for the HematoLogics Biomarker Diagnostic Panel and T-MRD panel at the desired MFI levels.
- The base particles exhibited forward scatter (FSC) and side scatter (SSC) characteristics comparable to lymphocytes, while the spike particles showed FSC and SSC profiles consistent with monocytes, as expected.
- Trucytes were distinguishable from biological cells and retained good staining quality without requiring protocol modification.
- In the era of precision medicine and targeted therapies, reliable quality control of specific and often rare antigens is increasingly essential. TruCytes allow standardized verification and quality assurance of these specialized clinical targets.