SLINGSHOT BIOSCIENCES

Application Note

TruCytes™ as a Reliable Alternative to PBMC Controls for Consistent Immunophenotyping in Flow Cytometry

Introduction

Flow cytometry-based immunophenotyping assays are widely used throughout the drug and diagnostic development life cycle to identify and quantify immune cell subsets, including T cells, B cells, and natural killer (NK) cells. These assays play a critical role in monitoring patient responses, evaluating therapeutic efficacy, and assessing potential safety concerns in preclinical research and clinical applications. However, their accuracy and reproducibility rely heavily on the quality and consistency of the control materials used.

Traditionally, donor-derived peripheral blood mononuclear cells (PBMCs) controls have been used for these assays, but they present significant challenges. These include the inherent biological variability between donors, lot-to-lot inconsistencies, limited stability, and complex sourcing logistics—all of which can compromise assay performance and delay development timelines. The logistical and ethical complexities of acquiring and maintaining human biological materials further complicate efforts to standardize and scale immunophenotyping workflows (Figure 1).

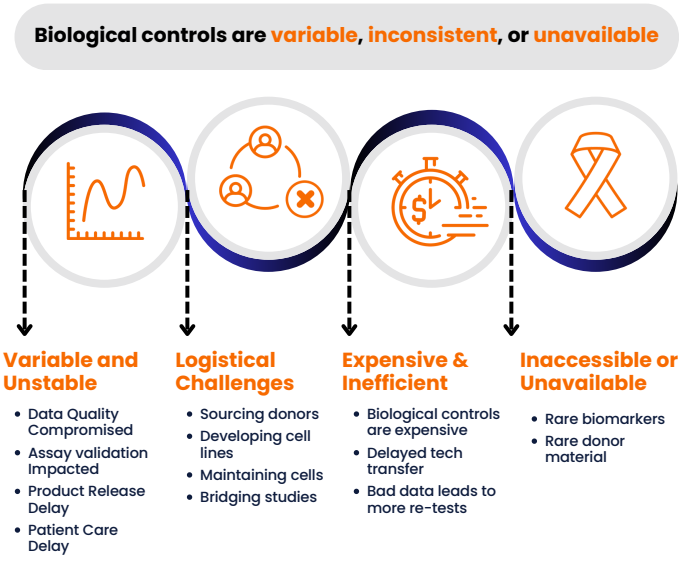

Figure 1. Drawbacks of Current Options for Cellular Controls: Cell Lines and Donor Material

To address these limitations, Slingshot Biosciences has developed TruCytes™ Lymphocytes Subsets Control. These novel cell mimics are engineered to replicate the optical and biochemical properties of PBMCs. By mimicking key immune cell subsets, such as T cells (CD3, CD4, CD8), B cells (CD19), NK cells (CD16, CD56), and monocytes (CD14), TruCytes™ provide a scalable, reproducible, and workflow-compatible alternative to donor-derived PBMC controls.

The proprietary technology behind TruCytes™ enables fine-tuning of both scatter coordinates (FSC and SSC) and biomarker expression, allowing the cell mimics to closely resemble the phenotype of primary immune cells in flow cytometry assays. Unlike PBMCs, which can vary in biomarker expression and population frequencies across donors, TruCytes™ are consistently manufactured to precise specifications to ensure enhanced reproducibility across assays, operators, sites, and instruments.

This application note presents data comparing TruCytes™ to traditional PBMC-based controls, demonstrating their lot-to-lot consistency, intra-lot reproducibility, extended stability, and compatibility with standard immunophenotyping assay protocols.

Methods

Study Design and Controls

This study evaluated the performance of TruCytes™ Lymphocytes Subset Control compared to traditional lyophilized and cryopreserved PBMC controls.

TruCytes™ are polymer-based lyophilized cell mimics engineered to replicate the optical and biochemical properties of PBMCs or leukopak-derived controls in terms of scatter, biomarker expression levels, and immune cell subset population frequencies. The populations included in TruCytes™ Lymphocyte Subset are T cells (CD3 and CD4/CD8), NK cells (CD16 and CD56), B cells (CD19), and Monocytes (CD14). All experiments were performed on a Cytek® Aurora CS flow cytometer.

Lot-to-Lot Consistency

The lot-to-lot consistency of TruCytes™ was assessed by comparing two lots of TruCytes™ Lymphocytes Subset Control with two lots of lyophilized and cryopreserved PBMC controls. Each lot was analyzed for variability in fluorescence intensity and population distribution, and the coefficient of variation (CV) was calculated to quantify variability. Intra-lot consistency was assessed by analyzing replicate samples run on the same instrument across different days and users.

Fixation Buffer Compatibility

The compatibility of TruCytes™ Lymphocytes Subset Control was evaluated with three commercially available fixation buffers: eBioscience™ IC Fixation Buffer, BioLegend® Fixation Buffer, and BD CytoFix®. After fixation, cells were stained with antibodies targeting immune cell markers (CD3, CD4, CD8, CD19, CD14, CD16, and CD56) to confirm the cell mimics retained their immunofluorescence capabilities. The ability of the fluorescent antibodies to detect their cognate antigens was evaluated based on signal intensity and antigen binding.

RBC Lysis Protocol Compatibility

To assess the compatibility of TruCytes™ Lymphocytes Subset Control with red blood cell (RBC) lysis protocols, two different RBC lysis methods were tested using a 1X RBC Lysis Buffer. Lyse/wash and lyse/no wash protocols were compared to a no-lysis control.

Antibody Clones and Fluorescent Labeling

TruCytes™ were stained with antibodies targeting CD45, CD3, CD4, CD8, CD19, CD14, CD16, and CD56. Each marker was evaluated using three different antibody clones to assess compatibility and effectiveness in differentiating positive and negative populations.

Data Acquisition and Analysis

Flow cytometry data were acquired using the Cytek® Aurora CS system. For each experiment, samples were run in triplicate, and data were collected for each immune cell subset of interest. Gating strategies based on forward scatter (FSC), side scatter (SSC), and fluorescence parameters were used to identify the immune cell subset populations. Data analysis was performed by calculating the CV for each control material to assess reproducibility and variability across lots and conditions.

Results & Discussion

Antibody Clone Compatibility of TruCytes™ Lymphocyte Subset Control

The TruCytes™ Lymphocyte Subset Controls were stained with commonly used antibody clones for each marker (Table 1).

Marker	Clone 1	Clone 2	Clone 3
CD45	2D1	HI30	MEM-28
CD3	SK7	UCHT1	OKT3/HIT3a
CD8	SK1	HIT8a	RPA-T8
CD4	SK3	OKT4	RPA-T4
CD16	B73.1	CB16	3G8
CD19	SJ25C1	HIB19	4G7
CD56	NCAM 16.2	MEM-188	MY-31
CD14	M5E2	61D3	HCD14

Table 1. Antibody clone compatibility. Note that clones 2 and 3 for all markers were tested using PE.

Each clone showed excellent separation between the positive and negative populations except for OKT3 and HIT3a for CD3. Based on these results, we recommend using the SK7 or UCHT1 clones for CD3 staining with TruCytes™ Lymphocytes Subset Control to ensure optimal performance in immunophenotyping assays. Figure 2 shows TruCytes™ Lymphocytes Subset Control stained with CD45 clones 2D1, HI30 and MEM-28.

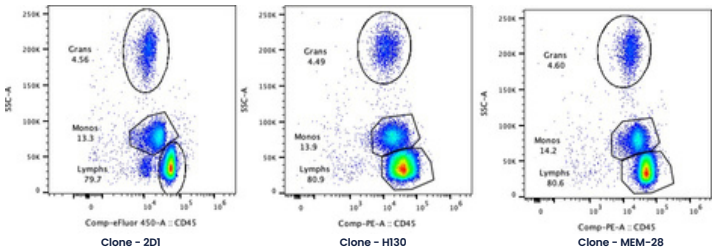

Figure 2. Flow cytometry plots showing TruCytes™ Lymphocytes Subset Control stained with CD45 clones a) 2D1, b) HI30, and c) MEM-28. All three clones demonstrated good separation between granulocyte, monocyte, and lymphocyte populations.

Lot-to-Lot Reproducibility

TruCytes™ Lymphocytes Subset Control can be manufactured with high reproducibility at full commercial scale. Eleven lots demonstrated %CV of 1.3% to 8.8% across all populations from scale up to manufacturing (Table 2).

Population Type	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Lot 10	Lot 11	CV%
Grans CD45+	5	4	5.8	4.8	4.7	4.8	4.5	5	5.3	5	4.8	8.81%
Monos CD45+	15.5	15.4	15.5	14.2	14.7	14.4	14.6	14	15.8	13.8	14.1	4.57%
Lymphs CD45+	78.7	79.1	77	80	79.6	80	79	80.3	77.3	79.8	79.7	1.32%
T cells CD3+	77.3	76.2	69.6	74.8	73.6	74.3	72.9	74.6	74	72.5	73.4	2.58%
B cells CD19+	12.5	13.7	16.5	14.5	15.7	14.8	16.2	14.7	15.1	16	14.4	7.51%
T Helper CD4+	67	65.4	65.3	68.2	66.1	66.5	65.4	66	66.2	66	67.9	1.40%
T Cytotoxic CD8+	33	34.6	34.6	31.7	33.8	33.5	34.6	33.8	33.8	33.9	32.1	2.76%
NKcells CD16+CD56+	94.2	99.8	98.8	99.8	99.9	98.8	99	99.7	98.8	98.8	98.6	1.53%
Classical Monos CD14+	93.6	99.8	98.6	99.3	98.6	94.1	98.4	97.7	99.5	99.3	99.5	2.10%

Table 2. TruCytes™ Lymphocytes Subset Control demonstrates high lot-to-lot reproducibility from scale up to manufacturing. %CV was calculated for the percent population across 11 lots.

The lot-to-lot consistency of TruCytes™ was further evaluated by comparing two lots of TruCytes™ with two lots of lyophilized and cryopreserved PBMC controls. The percent CV (coefficient of variation) for TruCytes™ ranged from 0.1% to 5.7%, compared to PBMCs, where CVs ranged from 1.6% to 36.6% (Table 3).

Population	Lyophilized PBMC Lots	Cryopreserved PBMC Lots	TruCytes™ Lymphocyte Subset Lots
Grans CD45+	N/A	N/A	2.5
Monos CD45+	13.9	34.5	4.4
Lymphs CD45+	10.2	15.2	0.6
T Cells CD3+	12.1	1.2	1.3
B Cells CD19+	36.6	19.8	5.7
NK Cells CD3-/CD19-	29.1	8.8	1.6
T Helper CD4+	15.2	17.4	1.2
T Cytotoxic CD8+	18.7	18.7	2.3
NK Cells CD16+/CD56+	17.7	3.3	0.3
Classical Monos CD14+	1.6	8.3	0.1

Table 3. TruCytes™ Lymphocytes Subset Control demonstrates less variation in % populations between lots than PBMCs. % CV was calculated for the percent population as evaluated on the Cytek Aurora across two lots of each different control material. Orange denotes high variability whereas blue indicates low variability.

TruCytes™ showed less variation in population percentages compared to PBMC-based controls, demonstrating their lot-to-lot consistency and suitability as more reliable and reproducible controls for flow cytometry assays.

Intra-Lot Sample-to-Sample Consistency

The intra-lot consistency of TruCytes™ was assessed across different users, instruments, and time intervals. The % CV across three replicate samples from the same lot ranged from 0.1% to 3.8%. When tested on the same instrument by the same operator on two different days, the % CVs were 0.3% to 3.8%. Analysis of the same sample by a single operator on two different instruments (Cytek Northern lights and Cytek Aurora) on the same day produced % CVs of 0.2% to 3.9%, while different operators assessing the same sample on the same instrument and day showed % CVs between 0.3% and 3.3% (Table 4).

Population	3 Replicates, Same Instrument and Day	Same Instrument and Operator tested on 2 Different Days	Same Operator and Day tested on 2 Different Instruments	Same Instrument and Day with 2 Different Operators
Grans CD45+	3.8	3.8	3.9	3.3
Monos CD45+	2.6	2.3	2.8	1.4
Lymphs CD45+	0.5	0.4	1.3	0.7
T Cells CD3+	0.2	0.4	0.2	0.3
B Cells CD19+	1.8	1.2	0.7	0.5
NK Cells CD3-/CD19-	1.2	2.1	1.6	1.7
T Helper CD4+	0.3	0.3	0.4	0.3
T Cytotoxic CD8+	0.6	0.5	0.7	0.6
NK Cells CD16+/CD56+	0.3	0.4	0.6	0.2
Classical Monos CD14+	0.1	0.2	0.2	0.2

Table 4. TruCytes™ Lymphocytes Subset Control shows intra-lot consistency. %CV was calculated for the percent population within the same lot of product to evaluate consistency between various users, instruments, and time intervals.

Overall, the high intra-lot consistency (within 4% CV) demonstrates that TruCytes™ can generate reliable immunophenotyping results with minimal variability.

Stability Analysis

We assessed the stability of TruCytes™ Lymphocyte Subsets Control using an accelerated stability study based on the Arrhenius equation (Table 5). Our results demonstrate that TruCytes™ remain stable for up to 18 months in their lyophilized format when stored at -20°C. This shelf life is comparable to cryopreserved PBMCs, which can be stored long-term under ultra-low temperatures, but it significantly exceeds the stability of fresh PBMCs, which are typically viable for only 24–48 hours post-isolation. Moreover, storage at -20°C eliminates the need for liquid nitrogen, which is required to maintain the viability and reproducibility of frozen PBMCs, offering a more convenient solution for long-term storage.

Population Type	Day 0 0 months Average	Day 7 6 months Average	Day 13 12 months Average	Day 20 18 months Average	Up to 18 months CV%
Grans CD45+	4.67	4.73	4.92	4.77	1.97%
Monos CD45+	14.64	14.43	14.84	14.76	1.05%
Lymphs CD45+	79.92	80.05	79.43	79.66	0.30%
T Cells CD3+	74.52	74.82	74.66	74.58	0.15%
B Cells CD19+	14.54	14.32	14.36	14.53	0.67%
NK Cells CD3-CD19-	10.92	10.84	10.97	10.89	0.42%
T Helper CD4+	68.02	67.84	68.13	67.86	0.18%
T Cytotoxic CD8+	31.97	32.15	31.85	32.13	0.39%
NK Cells CD16+CD56+	99.95	99.91	99.93	99.91	0.02%
Classical Monos CD14+	99.96	99.97	99.95	99.96	0.01%

Table 5: Accelerated stability was performed in triplicate for each time point using the Arrhenius equation. TruCytes Lymphocyte Subset Control was found to be stable for up to 18 months in the lyophilized form when stored at -20°C

Fixation Buffer Compatibility

Three commercially available fixation buffers (eBioscience™ IC Fixation Buffer, BioLegend® Fixation Buffer, and BD CytoFix™) were used to evaluate the compatibility with TruCytes™ Lymphocytes Subset Control for immunofluorescent staining. Following fixation, TruCytes™ Lymphocyte Subset Controls were stained with immune cell markers CD3, CD4, CD8, CD19, CD14, CD16, CD56, and CD45 (Figure 3) to confirm antigen binding.

TruCytes™ are compatible with commonly used antibodies and fixation buffers, allowing seamless integration into existing immunophenotyping workflows without the need for adaptation.

RBC Lysis Protocol Compatibility

Red blood cell (RBC) lysis is a standard step in immunophenotyping assays to eliminate contaminating RBCs while preserving lymphocyte integrity. Lyse/wash and lyse/no-wash protocols were compared to a no-lysis control using TruCytes™ to evaluate their impact on antigen binding and assay performance.

TruCytes™ Lymphocytes Subset Control demonstrates consistent performance in both lyse/wash and lyse/no-wash RBC lysis protocols, with no significant differences in immune cell subset separation or antigen binding (Figure 4). This provides flexibility in immunophenotyping assay design, allowing researchers to choose the RBC lysis protocol that best suits their workflow without compromising the quality or reproducibility of results.

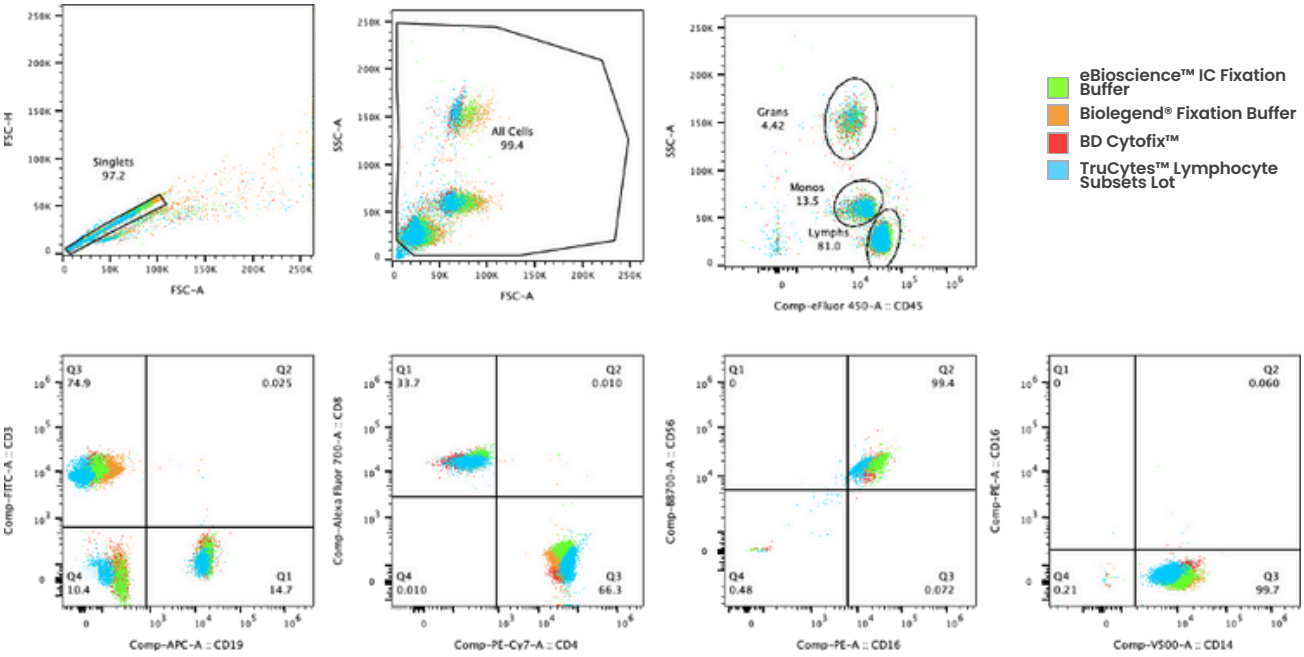

Figure 3. TruCytes™ Lymphocyte Subset Controls (Lot T4) stained with immune cell markers (CD3, CD4, CD8, CD19, CD14, CD16, CD56, and CD45) following fixation with three commercially available fixation buffers: eBioscience™ IC Fixation Buffer, BioLegend® Fixation Buffer, and BD CytoFix™. Flow cytometry plots showing distinct separation of immune cell subsets, including granulocytes, monocytes, and lymphocytes, with minimal shifts in marker expression and consistent antigen binding across all fixation conditions.

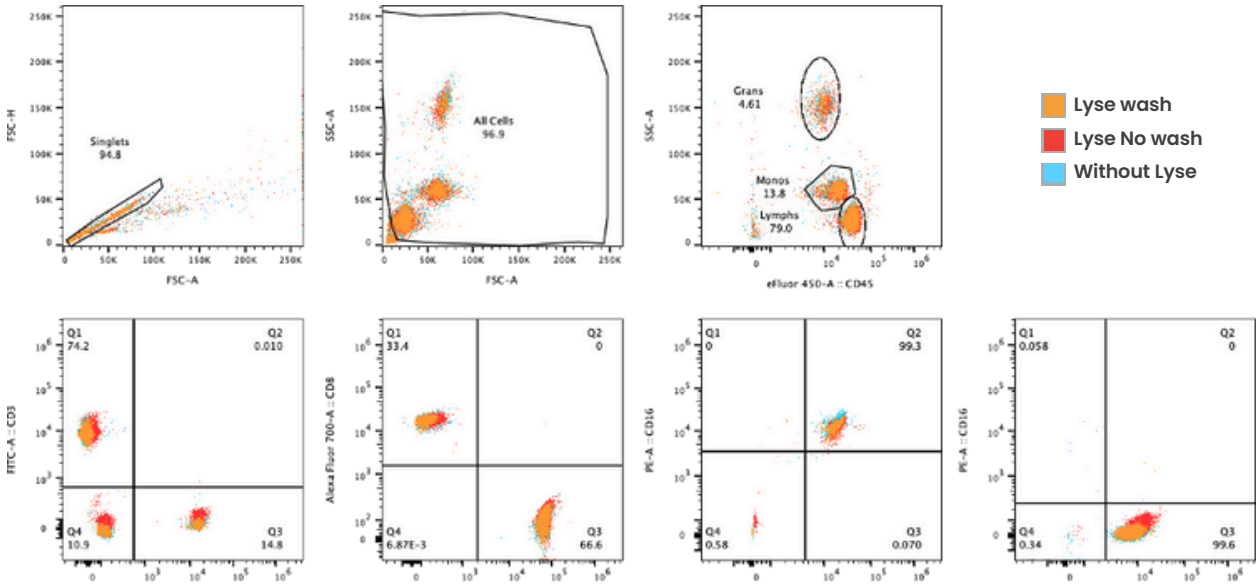

Figure 4. TruCytes™ Lymphocytes Subset Control stained with immune cell markers (CD3, CD4, CD8, CD19, CD14, CD16, CD56, and CD45) following lyse/wash, and lyse/no wash RBC lysis protocols. No significant differences in immune cell subset separation were observed between the RBC lysis protocols and the no-lysis control, indicating minimal impact on antigen binding and cell population identification.

Conclusion

TruCytes™ Lymphocyte Subsets Control provides a robust, reproducible, and ready-to-use alternative to PBMC-derived controls for flow cytometry-based immunophenotyping. This evaluation demonstrates that TruCytes™ deliver:

- Improved lot-to-lot consistency compared to PBMC-based controls, with TruCytes™ CVs ranging from 0.1% to 5.7% versus 1.6% - 36.6% CVs for PBMCs
- High intra-lot reproducibility, with CVs below 4% across users, instruments, and time intervals
- Closed-vial stability of 18 months at –20 °C, matching or exceeding cryopreserved PBMCs and significantly outperforming fresh PBMCs
- Broad compatibility with common antibodies, fixation buffers, and RBC lysis protocols
- Consistency at scale during manufacturing with a %CV range of 1.3% to 8.8% across all populations within 11 lots

Unlike PBMCs, which suffer from biological variability, degradation, and complex logistics and handling requirements, TruCytes™ cell mimics are engineered to deliver consistent marker expression and immune cell subset distributions across lots. These attributes—combined with long-term stability at –20 °C, scalable manufacturing, and broad workflow compatibility—make TruCytes™ better suited for research workflows, clinical trial assays, manufacturing processes, and quality systems requiring reliable, standardized controls.

By minimizing control-related variability, TruCytes™ improve data integrity, accelerate panel development, and support cross-lab reproducibility—streamlining both early-stage discovery and late-stage validation.

With flow cytometry increasingly integrated into clinical, manufacturing, and regulated environments, the need for consistent, high-quality assay controls is more critical than ever. TruCytes™ offer a better way forward: a reliable, reproducible, and scalable control that empowers scientists to move faster and with greater confidence.

From assay development to validation and regulatory submission, TruCytes™ help bridge the gap between innovation and implementation—enabling more efficient and reliable delivery of advanced therapies and diagnostics to the patients who need them most.

Ready to Purchase

Streamline your immunophenotyping workflows with TruCytes™ Lymphocytes Subset Control—reliable, ready-to-use, and built for consistent, reproducible results.

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