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WHITEPAPER

# Establishing Reliability of C-FREE™ Pluto Wash Technology Through Proven Laminar Wash™ Principles

## A Fluid Dynamics–Based Validation

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## Abstract

The Curiox C-FREE™ Pluto platform extends the proven reliability of Laminar Wash™ (LW) technology into an automated, pipette-driven format. We validate Pluto Wash™ (PW) as a centrifuge-less washing method grounded in shared fluid-dynamics principles. Comparative wash-out studies show equivalent dilution kinetics, cell recovery, staining quality, and reproducibility between PW and LW, with advantages over manual centrifugation in variability and debris carry-over. These findings support PW as a credible, scalable alternative for immunology, cell therapy, and clinical research applications.

## Contents



## 1. Introduction

Reproducible sample preparation is foundational to flow cytometry and single-cell assays. Centrifugation—still common practice—introduces variability, mechanical stress, and aerosol risk, which can undermine assay fidelity and comparability across sites (best-practice guidance emphasizes stringent controls and setup). Curiox Laminar Wash™ (LW) demonstrated that gentle, laminar buffer exchange can replace pelleting/resuspension, improving robustness of rare/fragile cell handling while maintaining staining performance. Pluto Wash™ (PW) carries these physics into standard labware via programmable pipetting and controlled buffer exchange cycles.

## 2. Shared Fluid Dynamics Principle

**Core concept:** Both LW and PW rely on laminar buffer exchange under controlled shear, rather than pelleting, to remove supernatant while keeping cells undisturbed at the bottom interface. What matters is interface fluid velocity, not container shape.

**Critical velocity threshold (~2 µm/s):** Internal derivations (Stokes-law–based settling estimates for PBMCs) established an interface velocity target on the order of ~2 µm/s; maintaining flow below this threshold preserves the quasi-static cell layer during aspiration/dispense.

**Consequence:** When this critical velocity is respected, cells remain settled while solutes and debris are exchanged, producing predictable dilution and minimal disturbance. PW achieves this in round wells/tubes by metering flow through disposable tips with programmed approach/withdrawal profiles; LW achieves it in wall-less plates by directing laminar streams over a flat interface. In both cases, cells are preserved—not pelleted.

## 3. Operational Equivalence of LW and PW

Despite different geometries, LW (flat, wall-less plate) vs PW (standard microtiter plate (MTP)/deep-well plate (DWP) with curved bottoms), both systems enforce the same interface-velocity constraint during aspiration/dispense, yielding equivalent washing physics.

- LW: precise laminar flow over a flat, wall-less surface.
- PW: gentle, pipette-driven exchange in Society for Biomolecular Screening (SBS) microplates/deep-well plates.

Because performance is determined by velocity control rather than well shape, the two implementations are operationally equivalent at the cell–fluid interface.

## 4. Methods

Experiments were performed side-by-side using the HT2100 Laminar Wash™ (LW) system and the Pluto Wash (PW) platform. PW was run at four programmed wash intensities (Light, Moderate, Heavy, Ultra), matched to the corresponding LW settings. Manual centrifugation (SOP) was included as a benchmark control.

**Sample matrices.**
Assays were conducted with human peripheral blood mononuclear cells (PBMCs) and lysed whole blood, chosen to represent both standard and challenging matrices for sample preparation.

**Readouts.**
Performance was evaluated by:

- Dilution kinetics of tracer (serum protein model) to assess wash efficiency,
- Cell recovery and viability,
- Flow cytometry–based subset frequencies ($CD45^+$, $CD3^+$, $CD4^+$, $CD25^+$, including regulatory T cells),
- Intracellular marker detection (Ki-67).

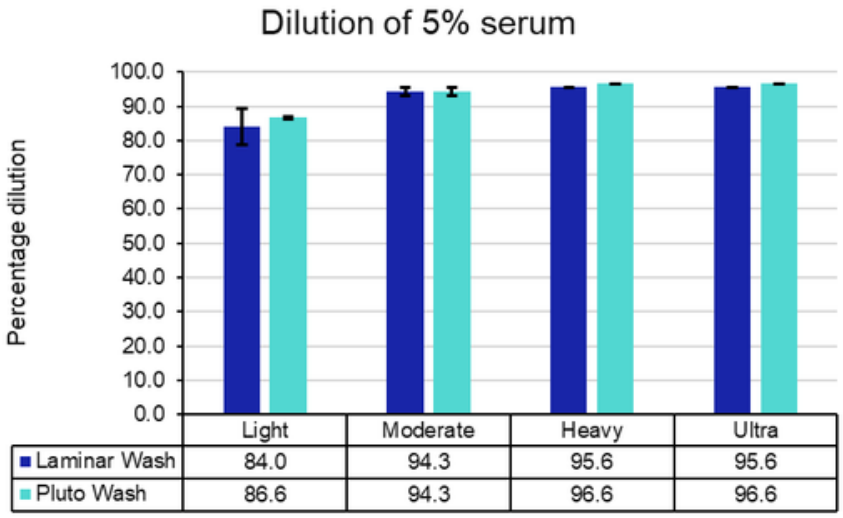
**Analysis.**
Subset gating followed standard lymphocyte hierarchies. Comparisons were made across LW, PW, and SOP platforms. Correlation was reported as coefficients of determination ($R^2$).

| Parameter | Laminar Wash (HT2100) | Pluto Wash (PW) | Manual SOP (MC) |
|---|---|---|---|
| Wash programs | Light → Ultra | Light → Ultra | Spin–decant |
| Sample types | PBMCs, lysed whole blood | PBMCs, lysed whole blood | PBMCs, lysed whole blood |
| Key readouts | Dilution kinetics, recovery, viability, staining, intracellular | Same as LW | Recovery, viability, staining |
| Replicates | ≥3 per condition | ≥3 per condition | ≥3 per condition |

# 5. Results

## 5.1 Wash-Out Kinetics

Both platforms show progressive dilution with increasing wash intensity (Light→Ultra). In R&D datasets, PW dilution curves overlap with LW, including in a challenging 5% serum matrix that increases viscosity/surface adsorption; this supports identical buffer-exchange physics independent of well geometry and determined by controlled interface velocity, supporting operational equivalence between the two platforms.

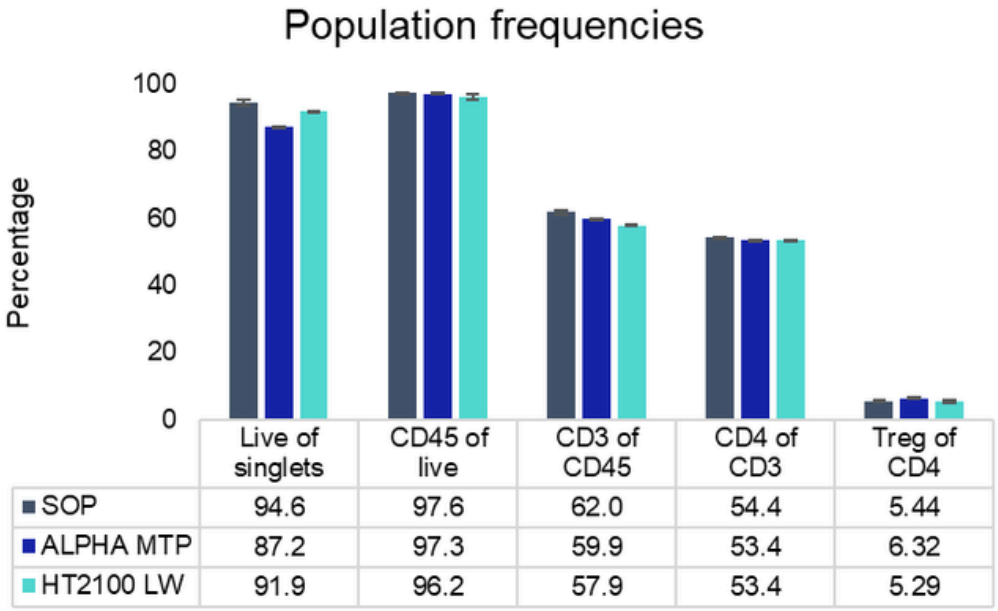



| | Light | Moderate | Heavy | Ultra |
|---|---|---|---|---|
| Laminar Wash | 84.0 | 94.3 | 95.6 | 95.6 |
| Pluto Wash | 86.6 | 94.3 | 96.6 | 96.6 |

**Figure 1.** Wash-out kinetics comparing HT2100 Laminar Wash and Pluto Wash across increasing wash intensities. The graph illustrates wash-out kinetics of a 5% serum solution, a deliberately challenging, protein-rich and viscous matrix. A 5% serum concentration is considered high and is known to impede complete washout because proteins increase viscosity and surface adsorption, slowing fluid exchange.

## 5.2 PBMC Recovery and Subset Resolution

Across lymphocyte gates (All Events → Live → CD45+ → CD3+ → CD4+ → CD25+), subset frequencies and resolution are comparable between LW and PW. Representative gates established with manual SOP are well-preserved with both LW and PW samples indicating comparable resolution resulting from efficient staining and washing. Quantitative analyses also demonstrate strong concordance, with correlation coefficient $R2 > 0.95$ for PW vs LW encompassing both major subpopulations and rarer regulatory T cells.




| | Live of singlets | CD45 of live | CD3 of CD45 | CD4 of CD3 | Treg of CD4 |
|---|---|---|---|---|---|
| SOP | 94.6 | 97.6 | 62.0 | 54.4 | 5.44 |
| ALPHA MTP | 87.2 | 97.3 | 59.9 | 53.4 | 6.32 |
| HT2100 LW | 91.9 | 96.2 | 57.9 | 53.4 | 5.29 |

**Figure 2.** Frequency comparisons for major lymphocyte subsets and regulatory T cells (CD45+, CD3+, CD4+, CD25+) across LW, PW (ALPHA), and manual centrifuge (SOP) methods.

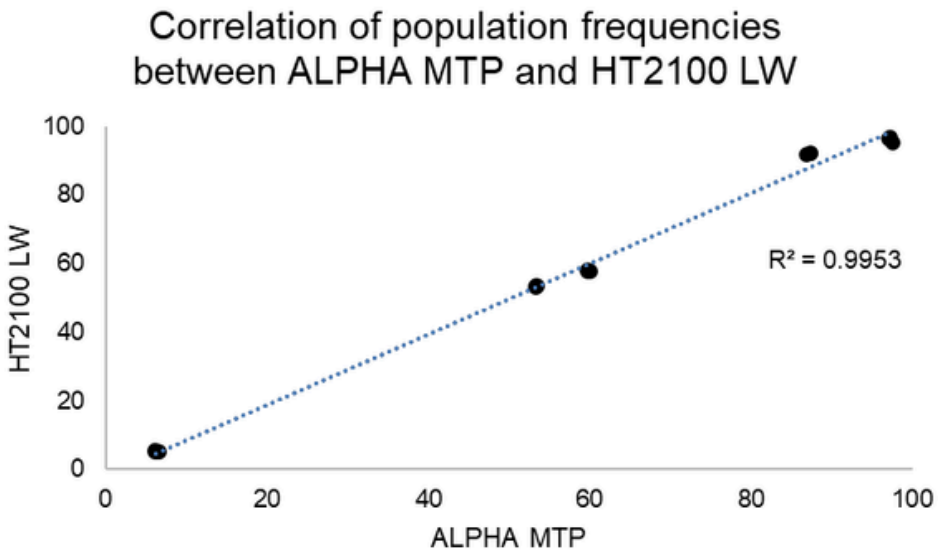



**Figure 3.** Correlation analysis between Laminar Wash and Pluto Wash. Scatter plot comparing subset frequencies across Laminar Wash (LW) and Pluto Wash (ALPHA). Each point represents the frequency of a lymphocyte subpopulation from matched samples. Linear regression analysis demonstrates excellent concordance, with $R^2 > 0.99$.

### 5.3 Intracellular Staining Performance and Background Control

Intracellular staining for Ki-67 demonstrated that Pluto Wash preserves assay fidelity, with background levels comparable to the manual centrifuge method and clear positive/negative separation across both CD4⁺ and CD8⁺ T cells. These results indicate that wash efficiency with Pluto Wash is sufficient to minimize nonspecific signal and support robust intracellular readouts, consistent with expectations from Laminar Wash–based workflows.

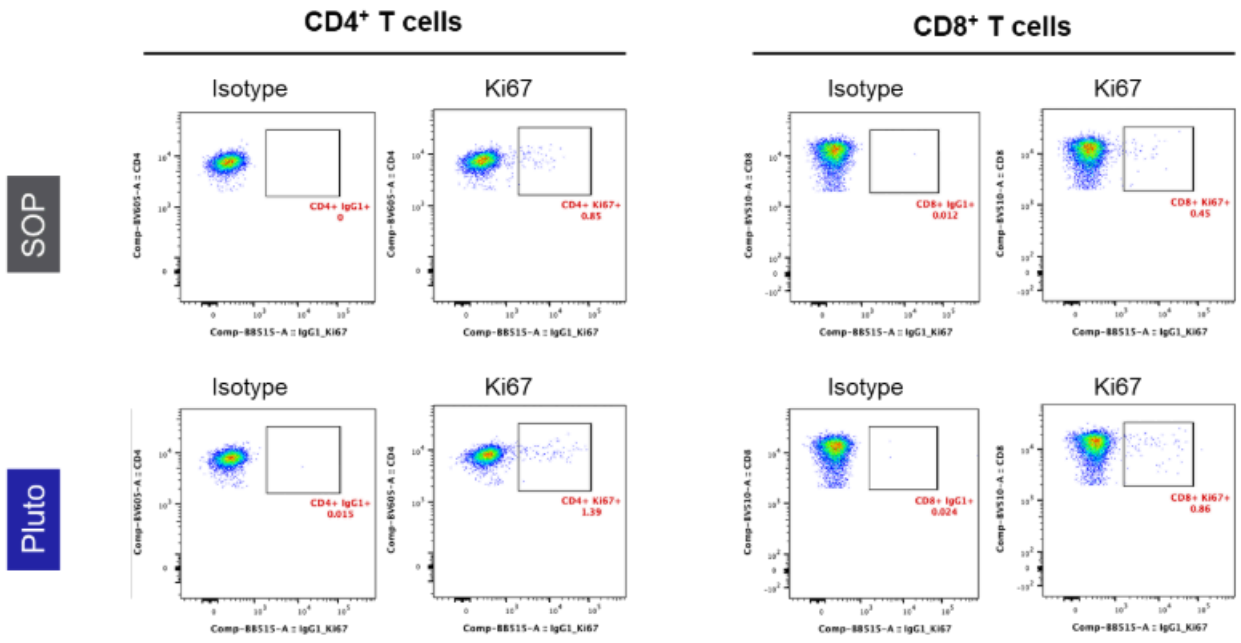



**Figure 4.** Comparable Data Quality: Ki-67 Background Staining. Representative plots comparing isotype control and Ki-67 staining for CD4⁺ and CD8⁺ T cells prepared using manual SOP and Pluto Wash. Both methods show similarly low background with isotype controls and clear separation of Ki-67–positive populations.

## 6. Data-Driven Validation: LW vs PW vs Manual Centrifugation (SOP)

**PBMC immunophenotyping:** It is widely adopted, data-rich, and representative of typical user workflows; comprehensive datasets exist across all three platforms (SOP, LW, PW).

**Comparative summary (typical ranges observed):**

| Metric / Parameter | Manual Centrifugation (SOP) | Laminar Wash (LW) | Pluto Wash (PW) |
|---|---|---|---|
| Cell Recovery (%) | Good (~70–90%) | Excellent (~90%+) | Excellent (~90%+) |
| Cell Viability (%) | Good, slightly variable (~80–90%) | Excellent (~90%+) | Excellent (~90%+) |
| Staining Quality (Index) | Good, slightly variable | Excellent, reproducible | Excellent, reproducible |
| Reproducibility | Moderate | Excellent | Excellent |

## 7. Discussion

The mechanistic determinant of wash performance is controlled interface velocity, not well geometry. Both LW and PW implement gentle, laminar buffer exchange that preserves the settled cell layer while exchanging supernatant; results converge across dilution kinetics, recovery, and staining quality.

**Key comparative findings:**

- Overlapping dilution curves (including high-protein matrices) indicate the same buffer-exchange physics for LW and PW.
- Subset frequencies and gating resolution align across SOP, LW, and PW, with strong linear correlation ($R^2 > 0.95$) between PW and LW.
- Intracellular Ki-67 staining shows similarly low background in isotype controls and clear separation of positive populations across SOP and PW, confirming that wash efficiency is sufficient to maintain assay fidelity for intracellular readouts.
- Staining quality and CVs: PW matches LW's SI/MFI with low intra-assay variability.

These findings are consistent with community guidance that emphasizes rigorous controls, standardized setup, and reproducible workflows for defensible cytometry data.

## 8. Conclusion

PW replicates LW's validated fluid-dynamics, delivering equivalent wash performance with the benefits of automation and standard labware compatibility. Evidence across PBMCs and whole blood supports operational equivalence and improved reproducibility vs. SOP. PW thus provides a credible path to scale centrifuge-less workflows without re-starting validation from scratch.

## References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