

Laminar Wash™ Performance of the C-FREE™ Pluto ALPHA System

Bead-Based Proxy for Cell Sample Automation

Executive Summary

The Curiox C-FREE™ Pluto Alpha System combines automated laminar washing with familiar 96-well microplate formats to deliver centrifuge-less sample preparation for flow cytometry and immunophenotyping. Its Laminar Wash™ plate technology enables:

- Gentle, reproducible cell or bead washing without centrifugation.
- Minimized sample and reagent consumption with low-volume washes (≤ 100 μL).
- Walk-away automation to reduce hands-on time and improve reproducibility.

To evaluate washing performance in a controlled and quantifiable way, 5–7 μm polystyrene beads were used as proxies for cells, reflecting the sedimentation behavior of lymphocytes and monocytes. Bead experiments allow safe, repeatable testing of wash efficiency, retention, and reagent removal—key predictors of real-world cell performance.

Scientific Rationale: Using Beads as Cell Proxies

- Bead size and density approximate human PBMCs and monocytes, enabling predictive assessment of laminar wash behavior.
- Advantages of bead-based testing:
 - Standardized geometry minimizes biological variability.
 - Facilitates visualization of wash flow dynamics.
 - Enables evaluation of multiple wash intensities and buffer volumes efficiently.

Translating bead results to cells:

- 5 μm beads $\rightarrow$ Lymphocytes
- 7 μm beads $\rightarrow$ Monocytes

By establishing recovery and wash performance using beads, labs can reliably predict retention for fragile or rare cell populations, minimizing experimental risk.

Laminar Wash Performance Data

Experimental Setup:

- Beads in volume of 25 μL per well.
- C-FREE™ Wash conditions: Moderate (automated aspiration and dispense cycles).
- Laminar Wash™ Wash conditions: 9 rounds, 5 $\mu\text{L/s}$
- Evaluation metrics: Bead retention %, supernatant clean-up using dye.

Representative Results:

- High Retention: Laminar Wash™ and C-FREE™ platforms achieve >90% retention with low CV (3% and 2%, respectively).
- Uniform Dilution: Trypan blue dilution across replicates (n=8) was uniform per platform, demonstrating consistent wash fluid exchange.
- Visual Indicator of Clean-Off: ALPHA washes exhibited lighter supernatant coloration, reflecting higher trypan blue clean-off rate.

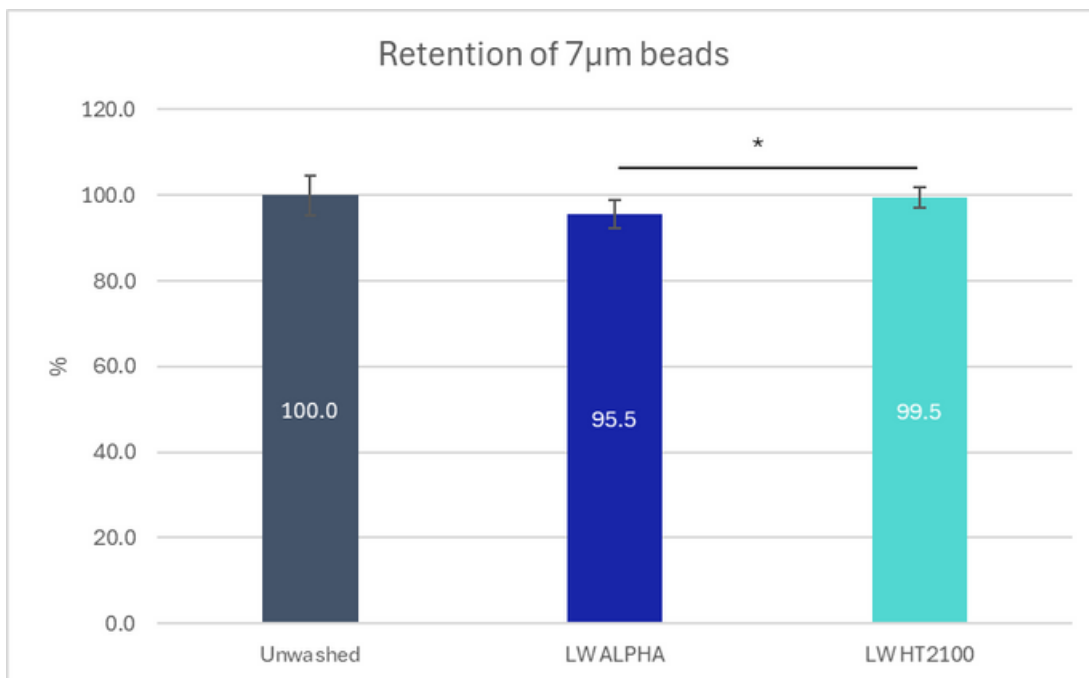


Figure 1. Comparison of bead retention between Laminar Wash™ and C-FREE™ technologies.

Interpretation:

- Both platforms deliver highly reproducible washes, minimizing sample loss.
- Clean-off efficiency, indicated by lighter trypan blue supernatant, highlights effective debris and reagent removal.
- Consistent dilution supports precision sample handling and predictable recovery.

Summary of Multi-Site Evaluations

Bead-based evaluations reliably predict cell performance in real-world immunophenotyping assays, as demonstrated in multiple Curiox conference presentations and online posters:

- Stanford University (AAI Conference Poster 2025) – Strong correlation to PBMC retention and improved staining indices versus manual centrifugation.
- BC Cancer (ICCS Clinical Flow Meeting Poster 2024) – Recovery and reproducibility comparable to conventional workflows, with reduced hands-on time.
- Whole Blood Immunophenotyping (SLAS Poster) – Clear memory T-cell subset resolution and low Ki-67 background with Pluto Alpha washes.

Together, these results confirm that C-FREE™ Pluto Alpha enables high recovery of fragile or rare populations, consistent reagent removal, and efficient sample and reagent use. Additional posters and supporting materials are available at <https://www.curiox.com/resources/posters>.

Contact us today to learn how Curiox C-FREE™ Pluto ALPHA to learn how the Pluto ALPHA can transform your workflows. www.curiox.com/pluto-alpha



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