TECHNICAL NOTE

LyoMax CyTOF Panel Validation for High-Quality Single-Cell Proteomics

Introduction

Why immune profiling?

Immune profiling is valuable for studying conditions in which the immune system plays a key role in disease pathology, progression or resolution. While immune profiling has emerged as a vital tool in the design and execution of clinical research, generating high-quality and reproducible data can be hindered by reagent reliability, stability and variability – factors that introduce risk and complexity into large-scale studies.

Value of standardized assays

Standardized CyTOF™ assays address these challenges by ensuring consistent performance in a dry single-tube format. These assays are stable across experimental conditions and eliminate the need for additional controls and titrations, significantly reducing technical variation and improving reproducibility and operational burden.

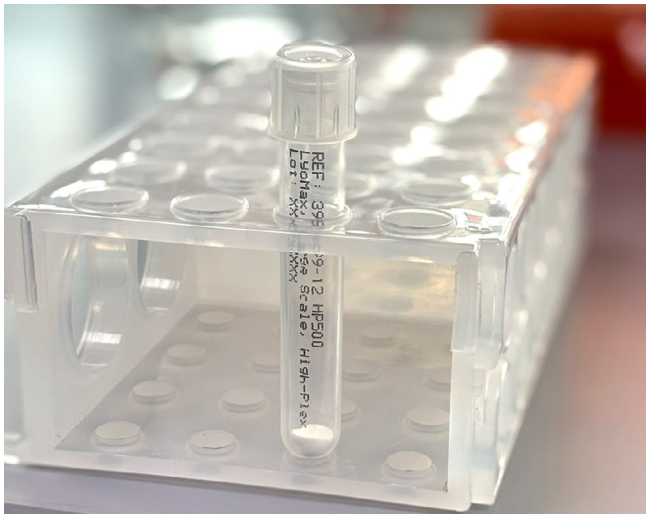
Advancing on the validated dry-format Maxpar™ Direct Immune Profiling Assay, custom lyophilized LyoMax™ Panels provide the same streamlined workflow but are individually tailored to specific markers of interest and standardized for large batches.

Purpose

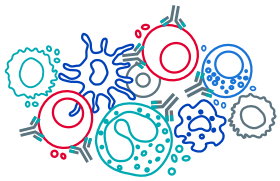
This technical note establishes lyophilized panel reliability, data reproducibility and experimental integrity across various workflows.

Summary:

- Dry-format lyophilized assays offer an extremely **stable and simple option** ideal for scaling multi-site and longitudinal clinical research programs
- Cross-validation of LyoMax CyTOF Panels in a liquid vs. lyophilized format demonstrates robust performance of lyophilized assays across **surface and intracellular markers**
- Validation testing on LyoMax CyTOF Panels confirms **robust precision** when used by different technicians and **high reproducibility** when barcoding, with i**mmense time savings** in sample prep and processing



LyoMax CyTOF Panels standardize immune profiling in a customized format



50-plus-marker capacity

- **Highest** parameters in a dry assay
- **Tailored** design with your markers of interest

Single dry format

- **No** liquid antibodies
- **Easy** staining workflow – just add samples

100-plus batch size

- **Standardized** lots for large batches
- **Reproducible** results across cohorts and sites

Figure 1. LyoMax Panels can include 50-plus isotope-tagged antibodies standardized in a dry single-tube format.

LyoMax CyTOF Panels are fully tailored, ready-to-use lyophilized assays, developed through the Standard BioTools™ Custom Lyophilization Service (Figure 1). LyoMax Panels are compatible with:

- Whole blood, PBMC and dissociated tissue
- Human and mouse samples
- Sample barcoding
- Stain-freeze workflows
- Stabilizing reagents

The panels are purpose-built to support large-scale, longitudinal and multi-site studies. These assay panels can be customized to program specifications, with the capability to monitor 50-plus markers simultaneously.

Assay design and testing process

The LyoMax Panel development process has undergone rigorous analytical validation using several parameters to evaluate assay performance across variable conditions, providing proof of specificity, sensitivity, stability, repeatability, reproducibility and precision.

In this technical note, we highlight three key LyoMax validation studies:

1. Lyophilized vs. liquid panel performance
2. Reproducibility
3. Compatibility with barcoding

Performance validation of surface and intracellular LyoMax Panels

To compare the performance of lyophilized antibodies to liquid antibodies in a surface staining panel, the Human TBMNK+G CyTOF Panel, 9 Antibodies (PN 201338) was lyophilized, and functional performance of the lyophilized panel was compared with liquid antibodies from the same lot using surface-stained PBMC (Figure 2a). Gated population frequencies and median intensities were comparable for all tested antibodies.

Next, to assess performance of a lyophilized intracellular panel as well as the compatibility of both surface and intracellular lyophilized panels in the same workflow, a subset of the Maxpar Direct T Cell Activation Expansion Panel (PN 201409) was evaluated alongside the lyophilized Maxpar Direct Immune Profiling Assay (PN 201334; Figure 2b). In both cases, liquid panels from the same antibody lots were compared with their lyophilized counterparts.

Results verify that accurate cell populations and comparable median intensities are detected in each condition (Figure 2), validating the robust performance of lyophilized assays.

Validation results demonstrate reliable functional performance of lyophilized antibody panels compared with liquid panels

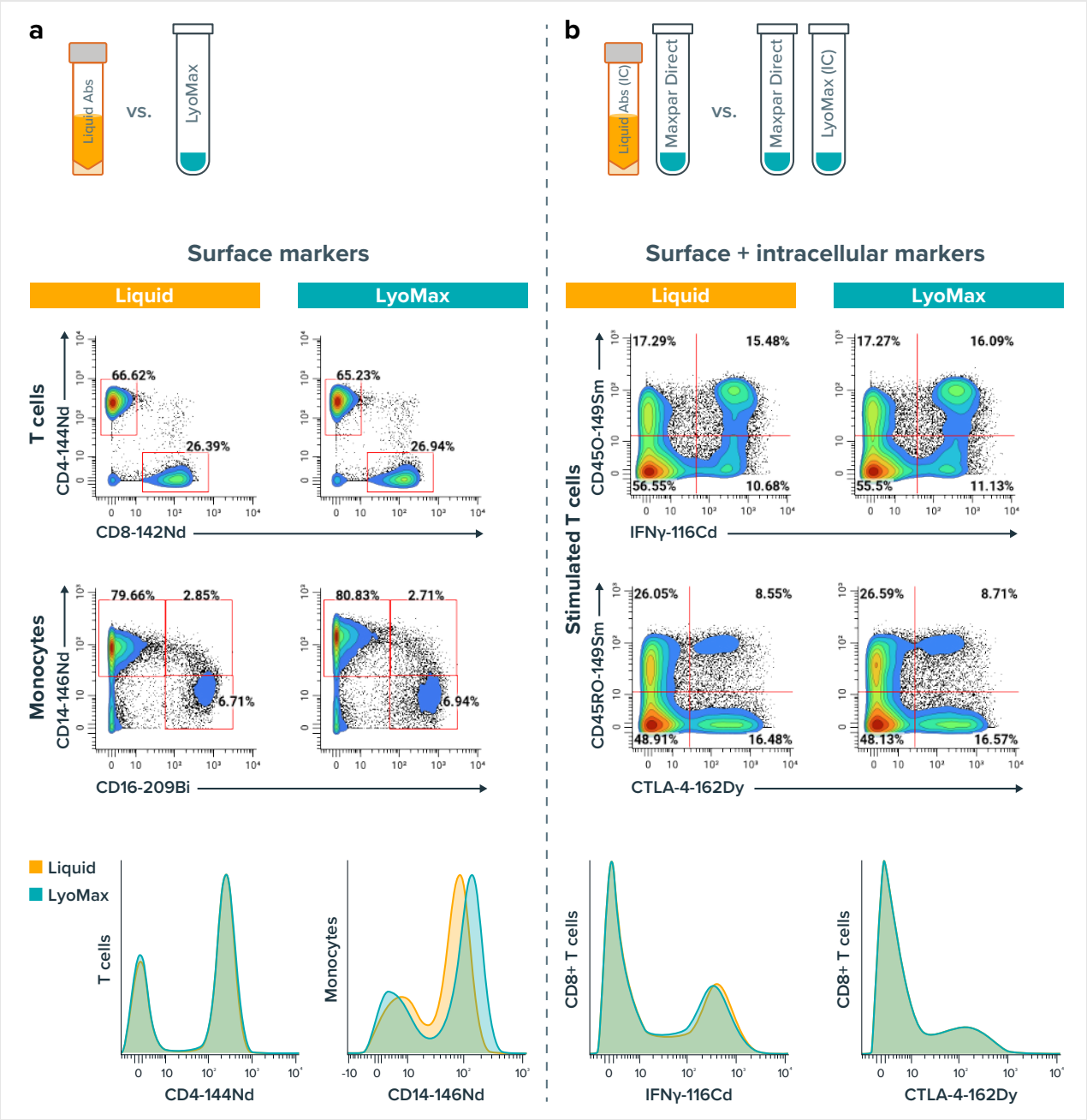


Figure 2. Lyophilized and liquid panels deliver comparable population detection and signal intensity. (a) Lyophilized surface panels show equivalent population frequencies and signal intensity compared with lot-matched liquid panels. (b) Similarly, lyophilized intracellular panels demonstrate consistent gating and intensity, confirming compatibility with lyophilized surface panels in a single workflow. For each cell population shown in the biaxial plots, liquid panels (left) and lyophilized panels (right) demonstrate precise detection across markers. Histograms confirm signal consistency between formats. Overall, LyoMax Panels enable an integrated staining workflow for both surface and intracellular assays.

Enhanced reproducibility across operators and replicates

Manual pipetting of liquid antibodies is a source of error that negatively affects experimental variability. To determine if using lyophilized antibody panels improves sample-to-sample and operator-to-operator variability, replicates from a 30-marker LyoMax Panel were compared with matched liquid antibodies from the same panel on stained PBMC samples.

Coefficient of variations (CVs) of median intensities derived from three operators independently pipetting three 30-plex liquid antibody cocktails were compared with CVs from three replicates of the lyophilized panel (Figure 3).

Both liquid and LyoMax antibodies showed minimal variation, with %CVs ≤5% for all markers (Figure 4a). Of the markers tested, 70% had lower CVs with the lyophilized panel compared with 30% with the liquid panel (Figure 4b). When averaged across all markers and operators, CVs were 2.66% for liquid panels vs. 1.66% for lyophilized panels (Figure 4c).

Overall, the lyophilized panel demonstrated lower variability than the equivalent liquid panel, demonstrating that lyophilized antibody panels reduce technical variation compared with liquid antibody cocktails across replicates and operators as measured by %CV.

Equivalent performance and low CVs across operators with LyoMax Panels

Figure 3. Assessment of the reproducibility of a lyophilized panel compared with a liquid panel in replicates of three, run by three technicians. Liquid antibody cocktail preparation and cell staining of PBMC samples was performed by three different operators, each preparing three independent technical replicates of antibody cocktails. Technical replicates were prepared in serial sequence, not in parallel, to mimic the potential noise introduced with each antibody cocktail preparation. The three different operators also stained cells with three technical replicates each of a single LyoMax Panel lot as a comparison.

Lyophilized panels consistently show improved reproducibility and precision over liquid panels

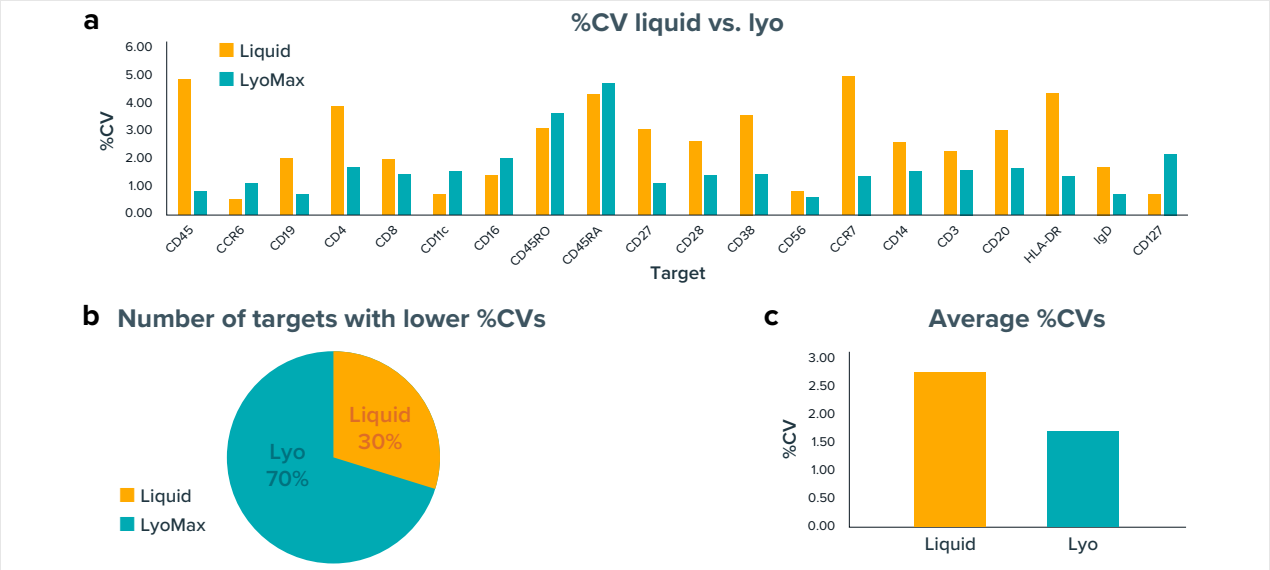


Figure 4. Lyophilized panels generate consistently lower %CVs than liquid panels, supporting improved reproducibility. (a) %CVs of markers from cell populations >5% of live single cells were included for comparison of liquid vs. lyophilized antibodies. (b) The number of antibodies with lower %CVs from either liquid or lyophilized panels were plotted in a pie chart. (c) %CVs from the liquid or lyophilized antibody panels were averaged and plotted in a bar chart. CVs were 2.66% for liquid panels vs. 1.66% for lyophilized panels.

Barcoding used with LyoMax Panels enables fast sample processing and reduces batch-to-batch variability

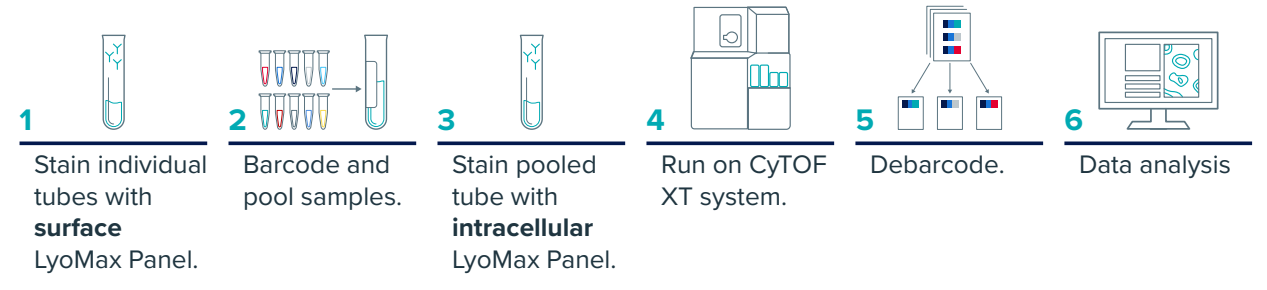


Figure 5. Lyophilized panels are compatible with barcoding workflows, generating reliable data from a stable dry format. Workflow: surface stain --> Pd barcode --> pool --> LyoMax IC stain --> acquire on CyTOF XT system

Barcoding compatibility

Barcoding reduces sample-to-sample technical variation by allowing multiplexed samples to be stained and acquired together, improving data consistency and workflow efficiency. To evaluate LyoMax Panel performance with a palladium barcoding workflow, surface-stained PBMC samples were barcoded using the Cell-ID™ 20-Plex Pd Barcoding Kit (PN 201060) and pooled into a single tube for intracellular staining and acquisition (Figure 5).

In parallel, PBMC samples were stained with the same surface and intracellular antibody panels without Pd barcoding (no barcode control). Frequencies of 30 gated populations for barcoded and non-barcoded samples were plotted, resulting in a coefficient of determination (R2) of 0.9879 (Figure 6). These results demonstrate that LyoMax Panels are fully compatible with Pd barcoding, enabling sample-sparing multiplexed workflows that minimize batch effects and streamline preparation, staining and acquisition.

Cell frequencies are highly correlated for each of the gated populations identified with or without sample barcoding

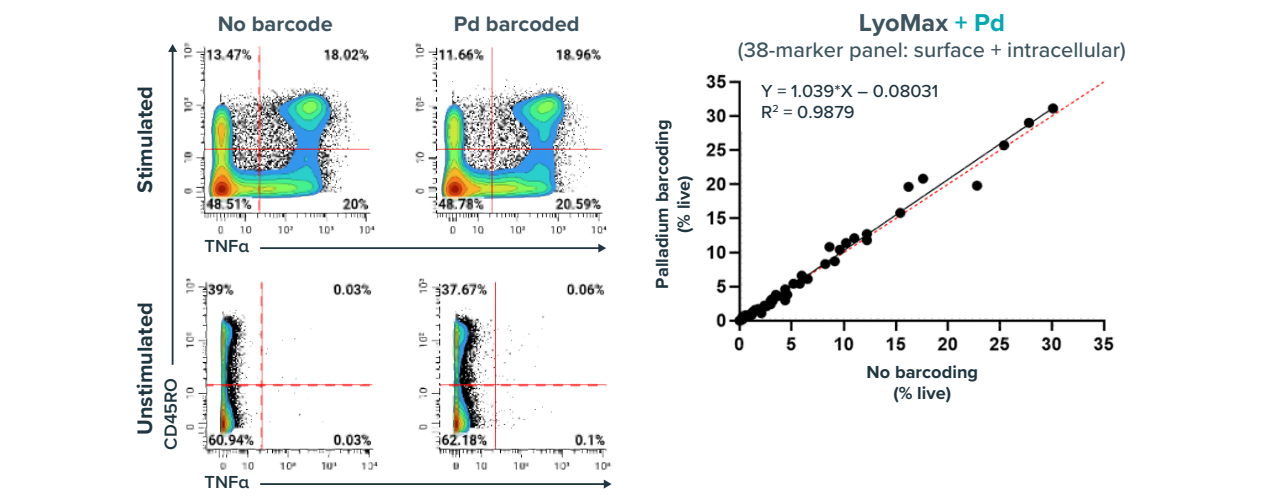


Figure 6. Accurate identification of cell populations using LyoMax Panels with the Pd-barcoding workflow is demonstrated with and without barcoding. Population frequencies (% live cells) were plotted for Pd-barcoded and non-barcoded samples. Correlation coefficients and equations are shown on the graph. Line of identity (slope = 1) is shown as a dotted line, and line of best fit for each condition is shown as a solid line.

Lyophilized panel validation process highlights minimal prep time needed, reducing time spent by more than two hours

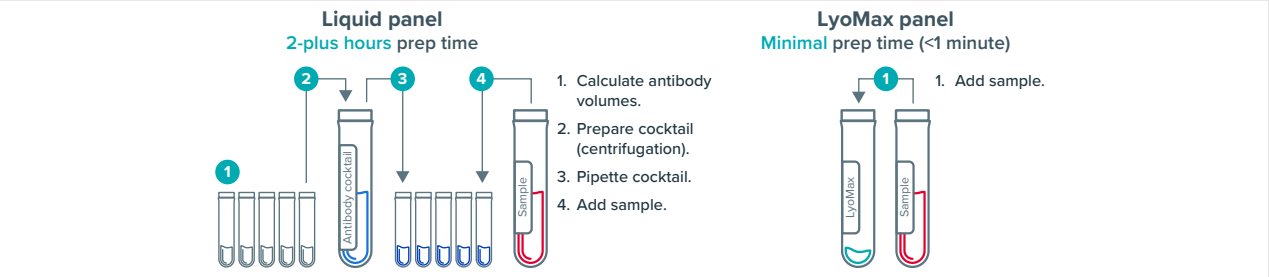


Figure 7. Lyophilized panels significantly reduce hands-on time by consolidating all antibodies into a single tube and eliminating the requirements for antibody volume calculation and cocktail prep. A comparison of the time taken for experiment prep using liquid panels and LyoMax Panels reveals a time savings of more than two hours.

Reduction in preparation time

During validation experiments, the time it took to prepare and pipette liquid and lyophilized panels was measured. Lyophilized panels reduce the time spent preparing an experiment by more than two hours based on a 30-marker panel (Figure 7). These dry-format LyoMax Panels eliminate manual preparation because the entire panel is contained in a single tube.

This removes the need for antibody volume calculations or pipetting steps required to make an equivalent antibody cocktail. Surface and intracellular LyoMax Panel staining can also be integrated into the same workflow for consolidation into a single assay for similar time savings.

Conclusions

Data presented in this technical note demonstrates that LyoMax Panels provide reproducible and precise quantitation of a broad range of immune cell populations and marker expression and are compatible with sample barcoding. In addition, the lyophilized panel format demonstrates superior accuracy in performance compared with the equivalent liquid panel as measured by %CV of marker signal intensities and consistency in cell population identification.

LyoMax CyTOF Panels are fully tailored, ready-to-use lyophilized reagents that offer the highest parameters available in a dry assay. The stable dry format eliminates the variability and manual preparation associated with liquid antibody cocktails and delivers reproducibility, long-term stability (>2 years shelf life) and simplified workflows. Available in large batches of standardized lots, LyoMax Panels are purpose-built to support large-scale, longitudinal and multi-site studies with consistency and ease.

Interested in custom LyoMax Panels? Learn more at **standardbio.com**

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Custom LyoMax Panels Validation Technical Note