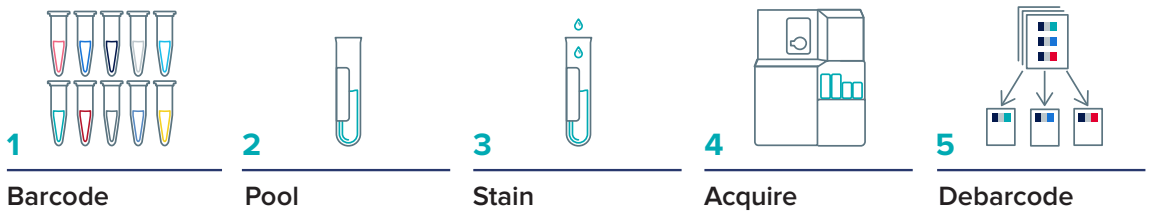
CyTOF™
Mass Cytometry

SAMPLE-SPECIFIC CELL BARCODING

Harmonizing large sample sets with powerful multiplexing

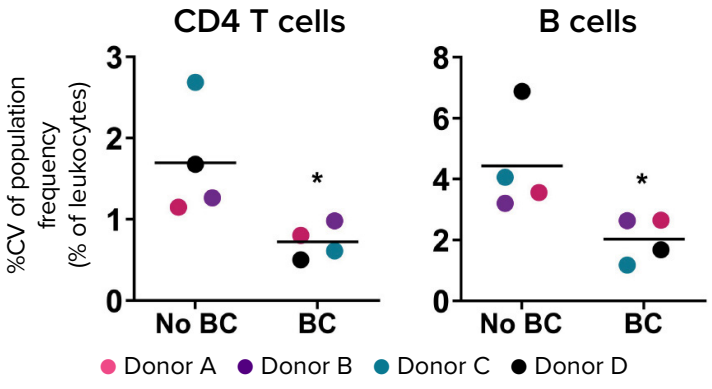
Cell barcoding is a proven strategy for multiplexing samples that minimizes technical variability – significantly improving reproducibility of immune phenotyping data across large studies.

Samples are tagged with a unique identifier, enabling combined staining in a single tube, eliminating staining variability and boosting sample throughput. Whether multiplexing fewer than 10 samples or more than 100, barcoding aids in scaling experiments while improving data consistency.



Workflow for barcoding. Individual samples are barcoded with unique metal isotopes for combined staining and acquisition, followed by debarcoding seamlessly integrated within CyTOF™ Software.

Improved sample-to-sample staining consistency



Barcoding minimizes sample-to-sample technical variation when multiplexed samples are stained and acquired together. Data from replicates of the same sample are compared between non-barcoded (No BC) and barcoded (BC) samples. See more.

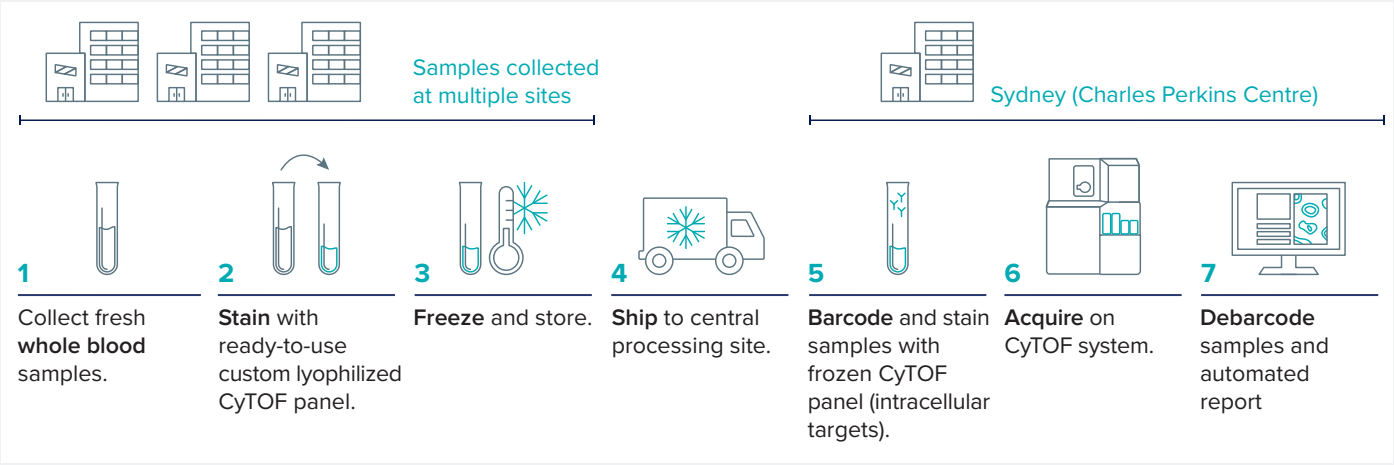
Benefits of sample multiplexing

- **Minimize** technical variability by harmonizing the workflow across all samples
- **Reduce** antibody/reagent use
- **Scale** experiments by multiplexing a large number of samples in a single tube
- **Proven** method used in 500-plus peer-reviewed publications

CASE STUDY

Increased workflow efficiency and data consistency in a longitudinal clinical trial

Researchers at the University of Sydney[1] developed a robust CyTOF workflow to overcome the limitations in remote sample collection quality. Sample multiplexing was leveraged to pool asynchronously collected samples in convenient batches for simultaneous intracellular staining, minimizing sample staining and acquisition variability.



This graphic highlights a highly reproducible and robust stain-freeze-ship CyTOF workflow with a 50-plus-marker functional and phenotypic panel to capture pre- and post-treatment responses across multiple sites. Sample barcoding was performed to multiplex 20 samples at a time.

Barcoding diverse cell types across a variety of research applications

Proven method used in 500-plus peer-reviewed publications

Publication highlights

A Phase 2 metastatic breast cancer clinical trial[2]

- Unique mass-tag cell barcoding with doublet-filtering scheme
- Paired **PBMC samples** collected before and after treatment were barcoded, reducing batch effects

A study of neuroinflammation in an EAE mouse model[4]

- Cell-ID™ palladium (Pd)-based barcoding
- Barcoded samples of **mouse spinal cord mononuclear cells** were combined in a single tube for staining and storage

126-plex barcoding of organoid samples[3]

- Thiol-reactive tellurium maleimide (TeMal) with cisplatin (Pt) barcoding
- Triplicates from 390 colonic **organoid cultures** were barcoded *in situ*

A trial on exercise effects in adults with prediabetes[5]

- Anti-CD45 palladium-based barcodes
- Paired **whole blood samples** were barcoded and stained together, reducing intra-patient technical sample variability

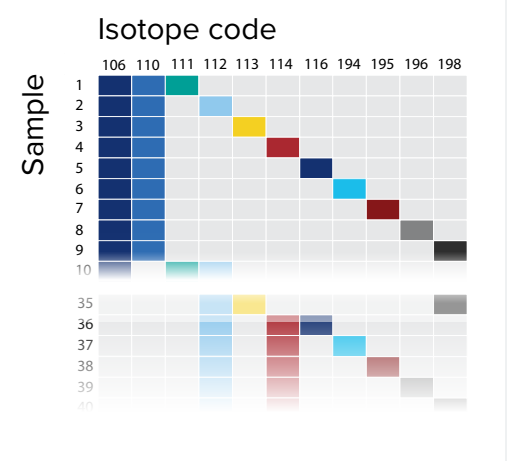
Choosing the right barcoding method that fits your needs

Novel advancements in barcoding reagents and methods enable researchers to select the ideal barcoding reagent and scheme tailored to their sample type, quantity and cell profiling needs for surface or intracellular targets.

	Palladium-Based Barcoding	Live-Cell Barcoding	Custom Barcoding
Cell type	Universal, cell-agnostic	Human leukocytes, CD45– cells excluded	Live or fixed cells
Advantages	• Ready-to-use kit to barcode up to 20 samples • Unique tags allow maximum panel size • Compatible with downstream surface, intracellular or intranuclear staining	• Multiplexing power for 35-plus samples • No fix/perm step required; fix-sensitive epitopes are preserved • Can be easily added to existing panels	• Allows barcoding of more than 100 samples for a single CyTOF experiment • Flexible selection of non-lanthanide tags: TeMal, selenium maleimide (SeMal), cisplatin (Pt), etc. • Can be easily added to existing panels
Considerations	Fix/perm step may require adjustments to panel/workflow	Barcoding scheme designed manually using software	Higher-plex (>100) barcoding schematic may not be feasible for all types of staining experiments
Product	Cell-ID 20-Plex Pd Barcoding Kit (PN 201060)	Maxpar™ anti-CD45 antibodies (cadmium and platinum isotopes)	Speak to your FAS to learn more about custom barcoding strategies
Resources	The Benefits of Palladium Barcoding on Data Quality and Workflow Application Note	Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry Application Note	Sufi, J. et al. *Nature Protocols* (2021). Seo, Y. et al. *Cytometry Part A* (2024)

Broad flexibility in panel design

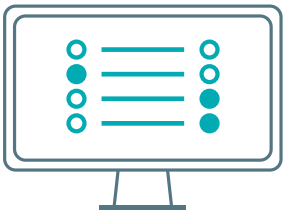
With the option to choose from various barcoding methods that use metal isotopes from outside the lanthanide series, barcoding can be easily added to an existing panel without compromising panel size.



Example custom barcoding design. A 50-plus-plex design using an 11-choose-3 live-cell barcoding scheme combining CD45 antibodies conjugated with cadmium (106, 110–114, 116) and platinum (194–196, 198) keeps remaining mass channels open for target analytes.

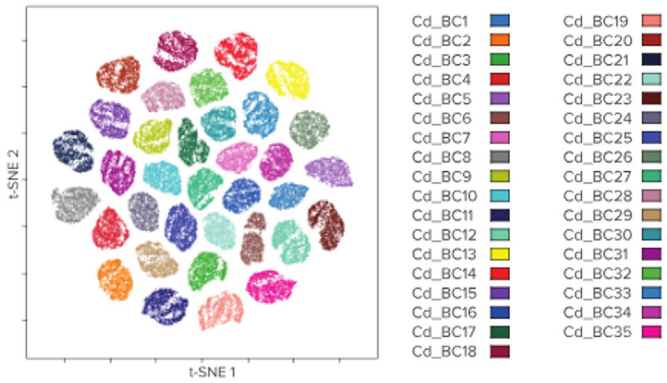
Advanced software to support sample multiplexing

Single-cell debarcoding software tools are available to debarcode samples into individual, cleaned-up files with improved doublet and multiplet discrimination.



CyTOF Software (v9.2)

- Debarcoder application optimizes analysis pipeline of multiplexed samples
- Integrated support for custom debarcoding of any isotope combination
- Increased number of barcoding schemes



Visualization of debarcoded samples via high-dimensional viSNE analysis. A 35-plex dataset of human PBMC samples barcoded with cadmium-labeled anti-CD45 antibodies is shown. Legend illustrates the colors displayed for each sample island. **See more.**

References

1. Smith, N.J. et al. "Comprehensive immunophenotypic strategies to enable equity in remote settings for improved cancer prognosis." Abstract 993, *Proceedings of the American Association for Cancer Research Annual Meeting. Cancer Research* (2024): 993.
2. Mo, H. et al. "Metronomic chemotherapy plus anti-PD-1 in metastatic breast cancer: a Bayesian adaptive randomized phase 2 trial." *Nature Medicine* 30 (2024): 2,528–2,539.
3. Qin, X. et al. "An oncogenic phenoscape of colonic stem cell polarization." *Cell* 186 (2023): 5,554–5,568.
4. Peruzzotti-Jametti, L. et al. "Mitochondrial complex I activity in microglia sustains neuroinflammation." *Nature* 628 (2024): 195–203.
5. Baker, C.J. et al. "Circulating CD31+ angiogenic T cells are reduced in prediabetes and increase with exercise training." *Journal of Diabetes and its Complications* 38 (2024): 108868.

Speak with a Technical Support Specialist about cell barcoding by visiting **standardbio.com/support**.

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Sample Multiplexing Cell Barcoding for CyTOF Flyer