APPLICATION NOTE

A 20-Marker Core Immune Flow Cytometry Panel Optimized for Flexible Whole Blood Preservation Workflows

Introduction

Phenotyping of immune cells in whole blood (WB) is critical for disease prognoses and monitoring the efficacy of therapeutic interventions. Accurate phenotypic analysis of freshly drawn blood is time sensitive and often poses a technical and logistical challenge due to the use of different sites for sample collection and analysis. Commercially available stabilization reagents preserve freshly collected WB, overcoming some of these challenges. However, phenotyping of preserved WB requires careful consideration, as some fixatives used in commercial stabilization reagents can impair antibody-epitope recognition of certain antibody clones, adversely affecting their cytometric detection.

The Standard BioTools™ Human Broad Immune Profiling CyTOF Panel, 20 Antibodies (referred to herein as the panel) is a CyTOF™ antibody panel identifying 32 immune cell types in fresh and preserved WB samples. The panel has been designed to be compatible with Proteomic Stabilizer PROT1 (Smart Tube Inc.) and Cytodelics Whole Blood Cell Stabiliser (Cytodelics) for high-parameter single-cell analysis of WB. The panel can be used as a part of several experimental workflows, which include stabilization/freezing of unstained or antibody-stained samples and sample multiplexing (Figure 1). This panel forms a core immunophenotyping panel upon which individual antibodies or other modularly designed panels can be added to achieve deeper immunophenotyping. This application note demonstrates the advantages of using CyTOF technology with this panel, providing a customizable and flexible approach for the immunophenotyping of WB from longitudinal, multi-site studies prominently employed in pharmaceutical and clinical research.

Objectives

This application note demonstrates the following key advantages of using the panel for immune profiling:

1. **Compatible with fixation/stabilization reagents** (PROT1 and Cytodelics) for analysis of WB, which allows for the:
   - Identification of prominent cell populations derived from preserved WB
   - Implementation of simplified WB processing workflows for increased staining flexibility
2. **Freezable antibody cocktail,** a unique feature of CyTOF technology
3. **Suitable for sample multiplexing.** Palladium (Pd) barcoding can be carried out on unstained or antibody-stained samples processed with stabilization reagents.

Human Broad Immune Profiling CyTOF Panel, 20 Antibodies

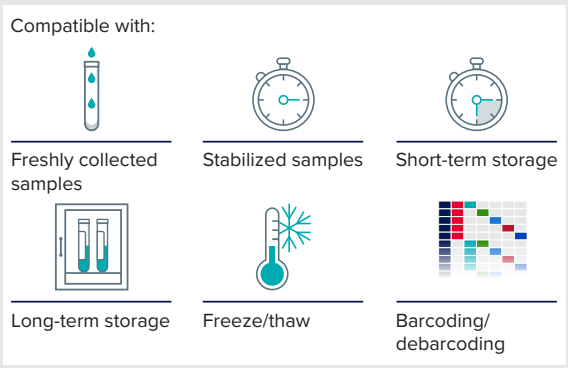


Figure 1. The Human Broad Immune Profiling CyTOF Panel, 20 Antibodies is fully compatible with staining of freshly drawn blood, PAXgene Blood DNA Tube for short-term storage (data not shown), PROT1 and Cytodelics stabilization reagents for long-term storage, freezing and thawing of antibody-stained samples[1], and sample multiplexing for the analysis of WB.

Study design

The panel comprises 20 markers enabling the identification of 32 immune cell types (Figure 2). Antibody clones and corresponding metal conjugates in this panel are listed in Appendix B. The panel antibodies were pooled to form an antibody cocktail and frozen (–80 °C) as single-use aliquots to be used for all staining reactions in this study.

WB from 3 independent donors was collected in BD Vacutainer EDTA Tubes. Fresh WB from each donor was processed using 3 experimental workflows (Figure 3).

Fresh-stain workflow

Fresh WB was stained with antibody cocktail, processed and acquired on the CyTOF XT system on the day of collection (Figure 3A).

Human Broad Immune Profiling CyTOF Panel, 20 Antibodies

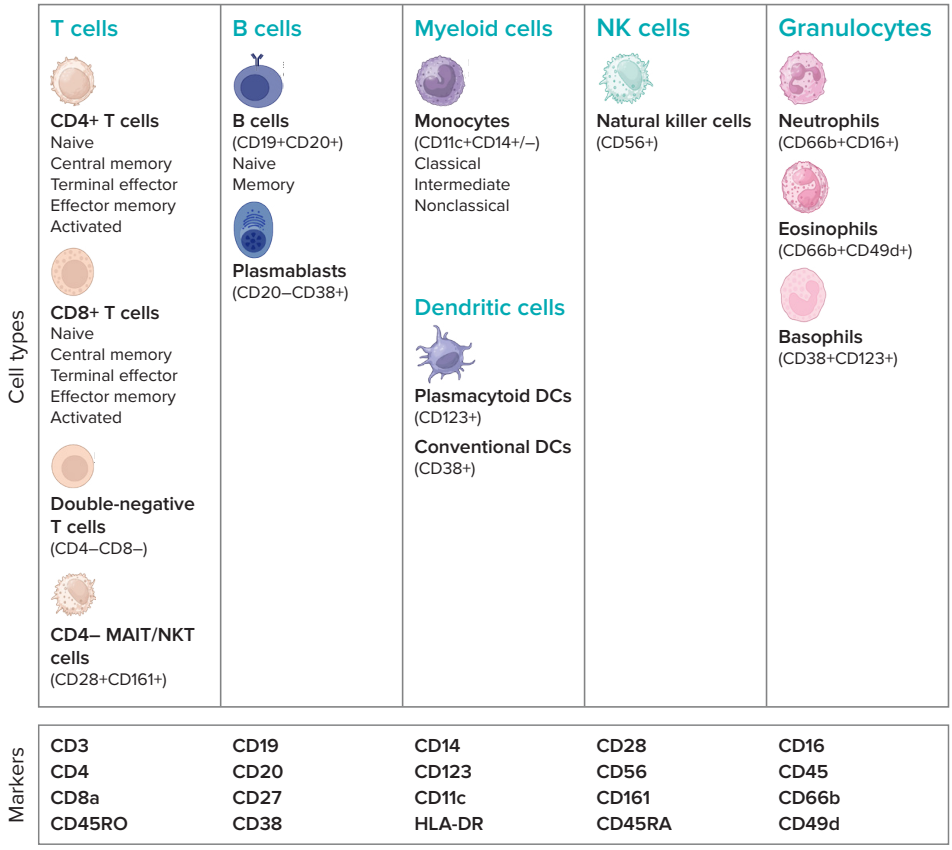


Figure 2. The Human Broad Immune Profiling CyTOF Panel, 20 Antibodies identifies 32 immune cell types comprised of major cell populations and rare cell types. The panel identifies all cell types shown above in WB. Peripheral blood mononuclear cells contain all cell types except neutrophils and eosinophils. Key markers used to define immune cell types are shown in parentheses. Refer to Supplementary Table 1 for the cell marker phenotypes of the 32 immune cell populations.

Stain-preserve workflow

Fresh WB was stained with antibody cocktail, stabilized with PROT1 or Cytodelics stabilization reagent, and preserved (–80 °C) on the day of collection. Subsequently, the samples were thawed, RBC lysed, Pd barcoded, pooled together and acquired (Figure 3B).

Preserve-stain workflow

Fresh WB was stabilized with PROT1 or Cytodelics stabilization reagent and preserved on the day of collection. Subsequently, the samples were thawed, RBC lysed, Pd barcoded, pooled together and stained with antibody cocktail before acquisition (Figure 3C).

WB samples from the 3 donors were frozen for different durations at the freeze step of the stain-preserve workflow and preserve-stain workflow: donor 3 for 7 days, donor 2 for 14 days and donor 1 for 21 days (Figure 3). The samples were then thawed and underwent downstream processing before acquisition, as shown in Figure 3B and 3C.

Additional experimental details can be found in the Methods section below. Ordering information for the reagents can be found in Appendix A and B.

Results

32 immune cell populations identified in fresh and preserved WB

The panel comprises 20 antibodies identifying surface-expressed markers for phenotyping of WB (Figure 2) and PBMC. Panel staining of WB processed through the 3 experimental workflows reveals 32 distinct immune cell populations in all samples (Figure 4 and Supplementary Table 2).

In all 3 experimental workflows, the panel identifies major cell populations, namely T cells, B cells, monocytes, dendritic cells (DCs), natural killer (NK) cells and granulocytes. Furthermore, rare cell types (for example, basophils and plasmacytoid DCs) and key subsets within major cell populations (for example, CD4+CD27+CD45RA+ naive T cells) are also identified (Figure 4). For all populations ≥5% in frequency, the %CV within each donor across the different workflows was <9.2%. Refer to Supplementary Table 2 for the frequencies of all 32 immune cell populations identified by the panel.

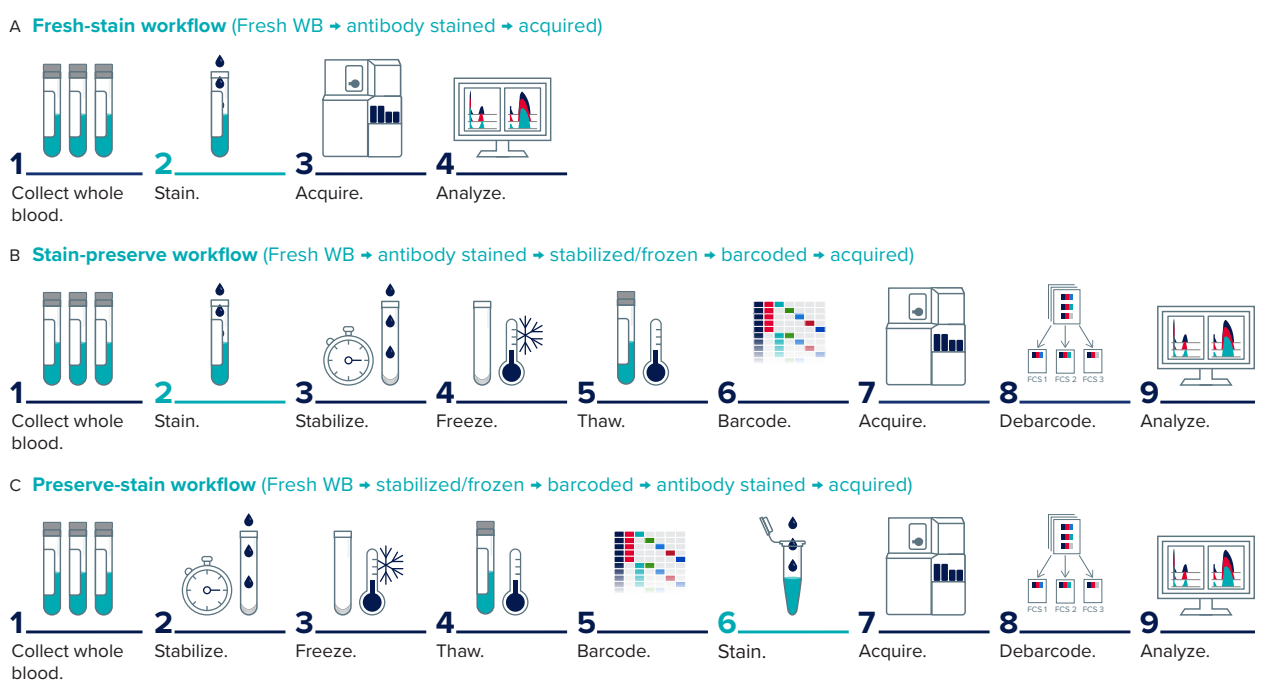


Figure 3. Flexible experimental workflows, including preservation with stabilization reagents and sample multiplexing, for the analysis of WB using the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies. WB from 3 independent donors was processed on the day of collection through the fresh-stain workflow (A) or processed with PROT1 and Cytodelics stabilization reagents in the stain-preserve workflow (B) and preserve-stain workflow (C) and frozen for 7 days (donor 3), 14 days (donor 2) and 21 days (donor 1). All the samples were thawed for downstream processing on the day of acquisition (B and C).

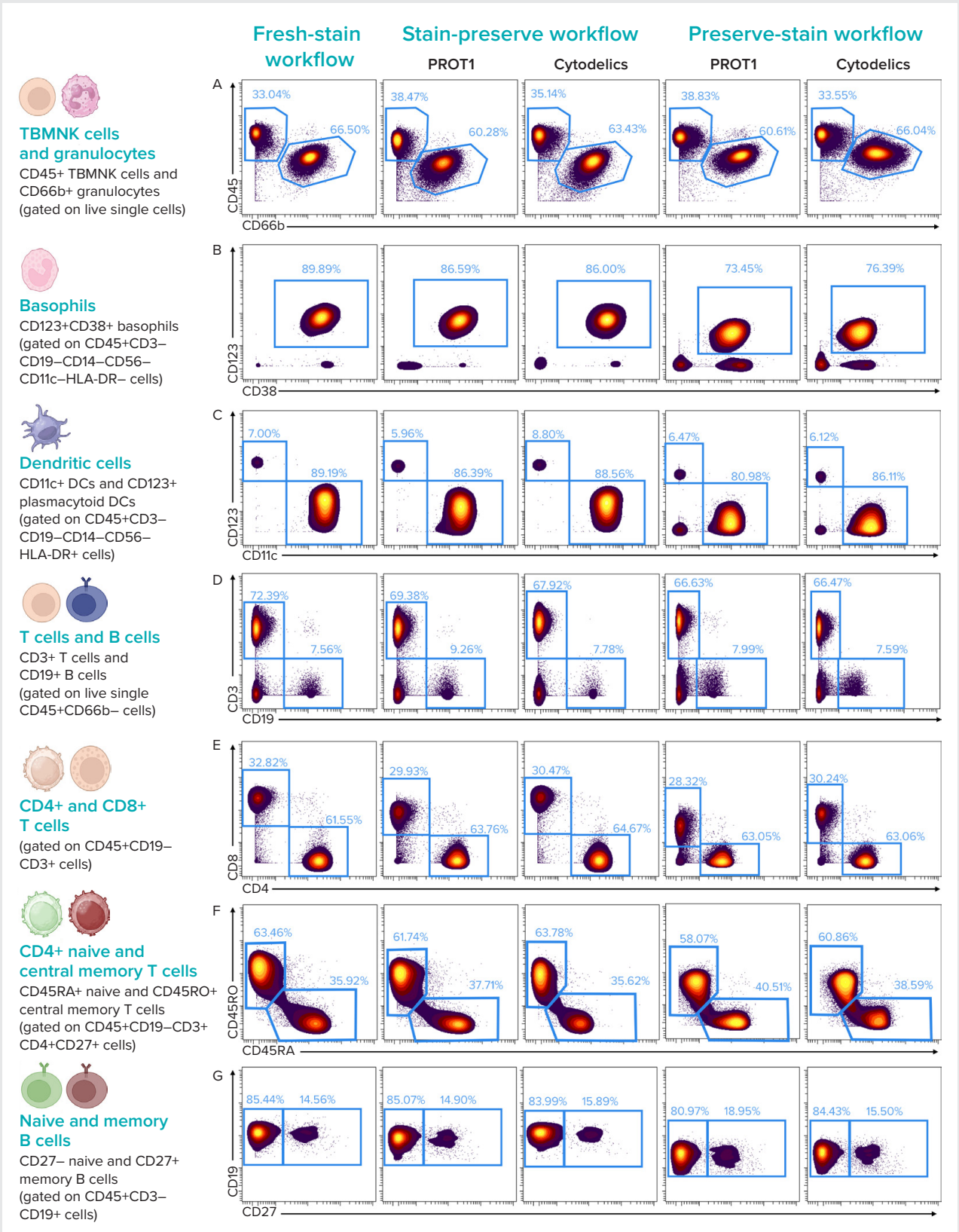


Figure 4. Representative dot plots showing the compatibility of the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies with PROT1 and Cytodelics stabilization reagents. These density dot plots are derived from traditional manual gating of CyTOF data using Cytobank Analysis software. Representative plots across different workflows and stabilization reagents are shown here. Population names, key markers, and parent population are shown to the left of the plots. Refer to Supplementary Table 1 for data from all 32 immune cell types identified by the panel.

Careful clone selection and metal assignment ensures that all 20 antibodies provide sufficient resolution for the gating of the population of interest in all 3 experimental workflows. Signal intensity of markers varies with the stabilization agent and experimental workflow; however, this variation does not compromise the gating of population of interest and population frequencies [for example, signal intensity from CD38 and CD123 defining basophils (Figure 4B)]. Processing samples with fixatives can give rise to nonspecific background signal in fluorescence-based flow cytometry[4,5]. The 20 panel antibodies do not show increased nonspecific binding when used with commercial stabilization reagents and sample multiplexing (for example, CD19 signal on CD3+CD19– T cells in Figure 4D)

Thus, the panel identifies 32 immune cell types and conserves the overall frequencies in WB processed through the 3 experimental workflows, overcoming the technical challenge of flow cytometric analysis of fixed cells in multi-site studies.

Discussion

This panel is designed to simplify single-cell high-dimensional analysis of PBMC and WB. In addition, this study highlights the advantages (Figure 1) of using this panel as a part of flexible workflows in conjunction with CyTOF technology for the immunophenotyping of blood.

Panel features

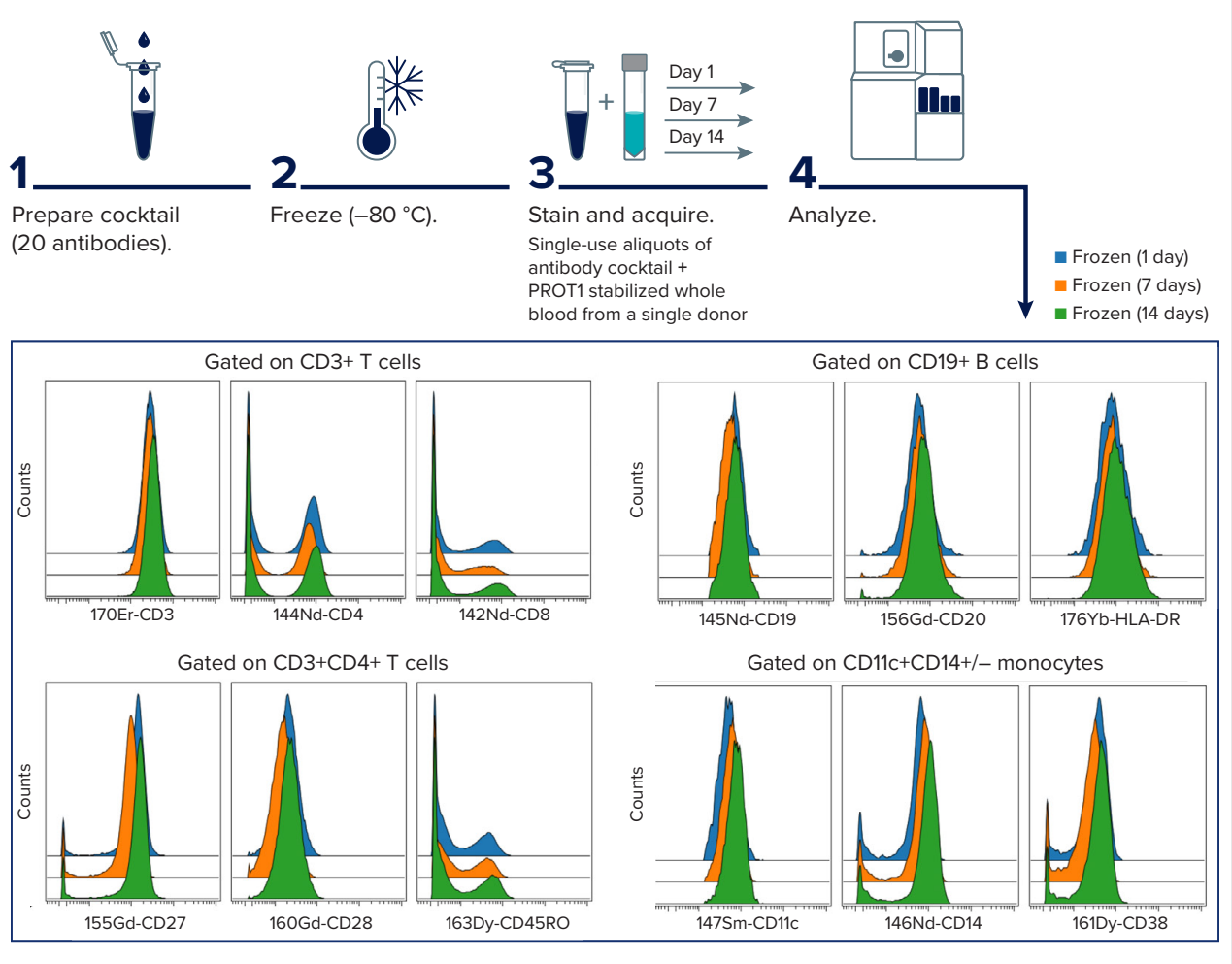
- **Designed for stabilized samples.** Careful antibody clone selection and metal assignment during panel design ensure that stabilization reagents (PROT1 and Cytodelics) do not influence the signal intensity of key markers in a way that affects the resolution of immune cell populations (Figure 4) and subsequent identification of 32 immune cell types. The panel also identifies 32 immune cell populations in WB stored in PAXgene Blood DNA Tubes up to 24 hr for short-term storage (data not shown).
- **Ready to be customized.** The panel utilizes only 20 channels for detection, leaving over 30 open channels for the addition of novel/unique markers, making the panel highly customizable to match the unique phenotyping needs of each user. For example, if the status of immune checkpoint proteins is of interest, compatible metal-labeled PD-1 and PD-L1 antibodies can be added to the panel (Supplementary Table 3).
- **Flexible panel design.** Based on the immune cell subset of interest (Figure 2) and study scope, a subset of the panel antibodies can be selected, thereby reducing the panel complexity without compromising performance. For example, if CD4+ naive T cells are of interest (Supplementary Table 1), panel antibodies can be selected for CD45+CD66b–CD19–CD3+CD8–CD4+CD27+CD45RO–CD45RA+ phenotype (Appendix B).
- **Freezable for improved data consistency.** Metal-tagged antibodies used in CyTOF workflows can be pooled as an antibody cocktail and frozen at –80 °C[6], as demonstrated in this application note. This unique feature of CyTOF systems can significantly improve data consistency in studies with repetitive staining/acquisition cycles spread over time or across different sites for blood collection and analysis (Box A).

Workflow features

The panel can be used with commercial stabilization reagents PROT1 and Cytodelics as a part of flexible experimental workflows for the analysis of WB (Figure 3). The workflows described in this study consist of permutations of staining, stabilizing, freezing, barcoding and acquisition steps and offer the following advantages:

- **Fresh staining flexibility.** Freshly drawn WB from different days can be stained with frozen antibody cocktail and acquired on the day of collection (Figure 3A) or cryopreserved to be acquired later[1].
- **Preservation flexibility.** Freshly drawn WB can be preserved with (stain-preserve workflow) or without (preserve-stain workflow) antibody staining before CyTOF analysis.
- **Barcoding flexibility.** Pd barcoding can be performed on stained samples processed with stabilization reagents and frozen (stain-preserve workflow) or on unstained preserved samples before antibody staining (preserve-stain workflow). Furthermore, Pd barcoding can be carried out before or after antibody staining in freshly stained samples[2]. Thus, several flexible multiplexing options are available for fresh and stabilized samples.

Box A: Frozen antibody cocktails are stable over time



Box A. Metal-conjugated antibodies in a frozen cocktail retain binding activity and yield comparable signal intensities over time. Panel antibodies were combined to make an antibody cocktail and frozen (–80 °C) as single-use aliquots. The frozen cocktail was thawed 1, 7 and 14 days after freezing and used to stain aliquots of PROT1 stabilized/frozen WB from a single draw of 1 donor to compare signal intensities of different markers over time. CyTOF data was analyzed by manual gating and the signal intensities of different markers in key immune cell populations are shown.

Freezing the metal-conjugated antibody cocktail for 1, 7 and 14 days yields comparable signal intensities for key markers. Preserved aliquots of PROT1 stabilized WB from a single draw of 1 donor were stained with frozen antibody cocktail on different days as a longitudinal control for signal intensity (Box A). Equivalent signal intensities were observed across the longitudinal control samples throughout the 14-day study. The bimodal distribution of events in markers showing a clear positive and negative peak (for example, CD45RO in CD3+CD4+ T cells) was similar for all frozen cocktail time points (Box A). The average %CV for the median signal intensity was 13.1% and ranged between 4.2–21.9%. These results suggest that antibodies from the panel can be pooled to make an antibody cocktail and frozen as single-use aliquots without loss of signal intensity over time. Frozen CyTOF antibody cocktails have been reported to be stable for up to 9 months[6]. This feature considerably improves data consistency by reducing pipetting errors, such as skipping an antibody completely or repeating the same antibody, introduced by individually prepared antibody cocktails.

Therefore, the ability to freeze the antibody cocktail made from this panel is valuable for generating reliable CyTOF data in longitudinal research and studies using different sites for collection and analysis of WB.

CyTOF features

CyTOF technology utilizes metal-labeled antibodies, resulting in discrete signals on a mass cytometer, with the opportunity to include more channels in the future. It offers distinct advantages over traditional fluorescence flow cytometry for immunophenotyping.

- **High-parameter, customizable panels.** CyTOF technology, with non-overlapping signals and a lack of autofluorescence, can measure 50-plus parameters per cell. A 20-marker panel can be conveniently used with CyTOF systems without the need for compensation and with over 30 open channels for panel customization.
- **Stable and reproducible signal.** Some fluorophores can be sensitive to fixation, freezing and storage, which adversely affects antigen detection by fluorescence flow cytometry in preserved samples. In contrast, samples stained for CyTOF workflows can be frozen and acquired later[1]. Additionally, the signal intensity derived from metal-labeled antibodies remains stable upon freezing and can be reproducibly detected over time (Box A), ensuring robust antigen detection by CyTOF technology in preserved samples.

Conclusion

The Human Broad Immune Profiling CyTOF Panel, 20 Antibodies simplifies single-cell high-dimensional analysis of WB and PBMC. This study highlights the versatile features of the panel and CyTOF technology for immunophenotyping (Figure 1).

- **Broad immune profiling.** The panel identifies 32 immune cell populations in fresh and preserved WB, broadly conserving population frequencies across workflows (Supplementary Table 2).
- **Flexible experimental workflows.** The panel can be used as a part of several flexible experimental workflows along with PROT1 and Cytodelics stabilization reagents, overcoming the logistical challenges of phenotyping WB processed by different collection and analysis sites.
- **Compatible with multiplexing.** Fresh or stabilized samples can be multiplexed before or after antibody staining, which provides the flexibility to reduce processing times and technical noise[2].
- **Frozen antibody cocktails.** Frozen antibody cocktails are unique to CyTOF systems[6], significantly improving data consistency in studies with repetitive staining/acquisition cycles spread over time (Box A).

In conclusion, the flexibility and features offered by the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies and CyTOF technology can serve as assets in longitudinal, multi-site studies prominent in clinical and pharmaceutical research.

Methods

Frozen antibody cocktail and signal intensity

Twenty antibodies from the panel were pooled to make an antibody cocktail and frozen at –80 °C as single-use aliquots[6] based on the experimental requirements. The frozen cocktails were thawed at room temperature (RT) and immediately used for staining cells. All staining in this study was performed using previously frozen antibody cocktails. Aliquots of PROT1 stabilized/frozen WB from a single draw of one donor were stained with the antibody cocktail on days 1, 7 and 14 after freezing as a longitudinal control to evaluate the performance of the frozen cocktail (Box A).

WB handling, staining, stabilization and downstream processing

WB from healthy adult donors was collected in BD Vacutainer EDTA Tubes. WB was stored at 4 °C and processed on the day of collection.

For the fresh-stain and stain-preserve workflows, WB was blocked with 300 kU/mL of heparin for 20 min. Heparin-blocked WB was added to the antibody cocktail (300 µL total staining volume) and incubated at RT for 20 min. Cell-ID™ Cisplatin-198Pt was added to the cells at a final concentration of 5 µM and further incubated for 10 min. The samples were then RBC lysed, washed with Maxpar™ Cell Staining Buffer (CSB) and fixed with fresh 1.6% formaldehyde (FA) solution before acquisition on the day of collection for the fresh-stain workflow. For the stain-preserve workflow, the antibody-stained samples were stabilized with PROT1 or Cytodelics stabilization reagents and frozen at –80 °C. The frozen samples were subsequently thawed, RBC lysed, washed with CSB, fixed with 1.6% FA and Pd barcoded. Refer to the Maxpar Cell Staining with Fresh Fix User Guide (FLDM-01319) for detailed staining instructions. The barcoded samples from different donors were pooled together and acquired as a single tube.

For the preserve-stain workflow, WB samples were incubated with Cell-ID Cisplatin-198Pt at a final concentration of 5 µM for 10 min at RT, stabilized with PROT1 or Cytodelics stabilization reagents, and frozen at –80 °C. The frozen samples were subsequently thawed, RBC lysed, washed with CSB, fixed with 1.6% FA and Pd barcoded [Refer to the Cell-ID 20-Plex Pd Barcoding Kit User Guide (PRD023) for detailed instructions]. The barcoded samples were pooled together, heparin blocked and stained with the antibody cocktail for 30 min at RT. The antibody-stained cells were washed with CSB before downstream processing.

Acquisition and analysis

Refer to the Maxpar Cell Staining with Fresh Fix User Guide for detailed pre-acquisition processing instructions. Briefly, antibody-stained samples from the different workflows were washed, incubated with iridium for 1 hr at RT, washed with CSB and Maxpar Cell Acquisition Solution Plus, filtered and counted. All samples in this study were acquired on a CyTOF XT system.. A total of 200,000–300,000 events were acquired at an acquisition rate between 250–300 events/sec.

Barcoded samples from the different donors were pooled together based on the workflow and stabilization agent used. Debarcoding was performed using CyTOF Software v8.1.0 to obtain individual FCS files.

The FCS files were evaluated for quality and consistency and analyzed by manual gating[3] using Cytobank Analysis software (cytobank.org). Median signal intensity of markers was extracted from cell populations that were derived from a terminal gate defined by that marker (for example, CD161 signal intensity was derived from CD4– NKT/MAIT cells falling in a CD161hi terminal gate; see Supplementary Table 1). Figure 2 and Figure 4 were adapted from illustrations generated using biorender.com.

Tips for success

- Calculate the amount of antibody needed based on the study design and prepare 10% excess cocktail. Freeze the cocktail as single-use aliquots. Depending on the selected workflow, PBMC and WB samples may require different volumes of antibody cocktail for staining.
- WB tends to stick to the sides of tubes and pipette tips. Prepare 20–30% excess volume of WB when heparin blocking so the required volume of WB is added to the antibody panel when staining.
- Expression of markers may vary depending on disease state and stimulation conditions. We recommend titration of the panel antibodies in the specific context of your application.
- We recommend carrying out pilot experiments to determine the experimental workflow and stabilization reagents that are optimal for the population of interest
- Follow the manufacturer's instructions for stabilization and RBC lysis using PROT1 and Cytodelics reagents
- Use Maxpar Water at room temperature for efficient RBC lysis when using Invitrogen Cal-Lyse Lysing Solution (Thermo Fisher Scientific)
- For maximum cell recovery, rinse each individual sample tube after combining the barcoded samples
- Scale up buffers and antibody volumes according to cell number as described in the Cell-ID 20-Plex Pd Barcoding Kit User Guide
- We recommend using iridium at 12.5–25 nM concentration. However, users should titrate the iridium concentration needed in their specific experimental context.

Appendix A: Ordering information

Standard BioTools Reagents	Part Number
Human Broad Immune Profiling CyTOF Panel, 20 Antibodies*	201339 B/C†
Maxpar Cell Surface Staining Kit: • Cell-ID Intercalator-Ir – 12.5 µM • Maxpar Cell Acquisition Solution Plus – 200 mL • Maxpar Cell Staining Buffer – 500 mL • Maxpar PBS – 100 mL • Maxpar Fix and Perm Buffer – 25 mL	201601
Maxpar Cell Acquisition Solution Plus for CyTOF XT – 1,000 mL	201244
Cell-ID 20-Plex Pd Barcoding Kit	201060
Cell-ID Cisplatin-198Pt	201198
Maxpar Water – 500 mL	201069

* Refer to Appendix B for ordering information of individual antibodies in the panel.

† Part numbers end in B for 100 µL per tube and C for 25 µL per tube.

Appendix B: Antibody products in the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies

Cell Population	Marker	Clone	Metal	Part Number†
Pan-leukocyte	CD45‡	HI30	89Y	3089003
T cell	CD8a‡	SK1	142Nd	3142021
	CD4‡	SK3	144Nd	3144029
	CD45RA	HI100	150Nd	3150034
	CD161	HP-3G10	151Eu	3151029
	CD27	L128	155Gd	3155001
	CD28	CD28.2	160Gd	3160003
	CD45RO	UCHL1	163Dy	3163032
	CD3‡	UCHT1	170Er	3170001
B cell	CD19‡	HIB19	145Nd	3145020
	CD20	2H7	156Gd	3156037
Myeloid	CD123/IL-3R	6H6	143Nd	3143014
	CD14‡	M5E2	146Nd	3146022
	CD11c	Bu15	147Sm	3147008
	CD38	HB-7	161Dy	3161034
	HLA-DR	L243	176Yb	3176027
	CD16‡	3G8	209Bi	3209002
NK cell	CD56‡	NCAM16.2	149Sm	3149021
Granulocyte	CD66b‡	G10F5	152Sm	3152019
	CD49d	9F10	141Pr	3141004

† Part numbers end in B for 100 µL per tube and C for 25 µL per tube.

‡ Nine antibodies from this panel form the Human TBMNK+G CyTOF Panel, 9 Antibodies (201338). Refer to the Human TBMNK+G CyTOF Panel, 9 Antibodies Technical Data Sheet (TDS-00727) for details.

Supplementary Table 1: Population gating table for 32 immune cell populations

Gates used to distinguish cell populations stained by the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies. All listed cell populations should also include the cleanup gates[3] (not shown in this study).

No.	Name	Phenotype
1	CD66b+ (neutrophils and eosinophils)	CD45loCD66b+
2	Neutrophils	CD45loCD66b+CD49d–CD16hi
3	Eosinophils	CD45loCD66b+CD49d+CD16lo/–
4	CD45+ (TBMNK)	CD45+CD66b–
5	Basophils	CD45+CD66b–CD3–CD19–CD14–CD56–CD11c–HLA-DR–CD123+CD38+
6	Dendritic cells (DCs)	CD45+CD66b–CD3–CD19–CD14–CD56–HLA-DR+
7	Plasmacytoid DCs	CD45+CD66b–CD3–CD19–CD14–CD56–HLA-DR+CD11c–CD123+
8	CD38+ conventional DCs	CD45+CD66b–CD3–CD19–CD14–CD56–HLA-DR+CD11c+CD38+
9	Monocytes	CD45+CD66b–CD3–CD19–CD123–CD56–HLA-DR+CD11c+CD14+/–CD11chi
10	Classical monocytes	CD45+CD66b–CD3–CD19–CD123–CD56–HLA-DR+CD11c+CD14+CD16–
11	Transitional monocytes	CD45+CD66b–CD3–CD19–CD123–CD56–HLA-DR+CD11c+CD14+CD16+
12	Nonclassical monocytes	CD45+CD66b–CD3–CD19–CD123–CD56–HLA-DR+CD11c+CD14–CD16+
13	Natural killer cells	CD45+CD66b–CD3–CD19–CD14–HLA-DR–CD45RA+CD56+
14	Total B cells	CD45+CD66b–CD3–CD19+
15	Naive B cells	CD45+CD66b–CD3–CD19+CD27–
16	Memory B cells	CD45+CD66b–CD3–CD19+CD27+
17	Plasmablasts	CD45+CD66b–CD3–CD19+CD27+CD20–CD38+
18	Total T cells	CD45+CD66b–CD19–CD3+
19	CD4+ T cells	CD45+CD66b–CD19–CD3+CD8–CD4+
20	CD4+ naive T cells	CD45+CD66b–CD19–CD3+CD8–CD4+CD27+CD45RO–CD45RA+
21	CD4+ central memory T cells	CD45+CD66b–CD19–CD3+CD8–CD4+CD27+CD45RA–CD45RO+
22	CD4+ effector memory T cells	CD45+CD66b–CD19–CD3+CD8–CD4+CD27–CD45RA–CD45RO+
23	CD4+ terminal effector T cells	CD45+CD66b–CD19–CD3+CD8–CD4+CD27–CD45RO–CD45RA+
24	Activated CD4+ T cells	CD45+CD66b–CD19–CD3+CD8–CD4+CD38+HLA-DR+
25	CD8+ T cells	CD45+CD66b–CD19–CD3+CD4–CD8+
26	CD8+ naive T cells	CD45+CD66b–CD19–CD3+CD4–CD8+CD27+CD45RO–CD45RA+
27	CD8+ central memory T cells	CD45+CD66b–CD19–CD3+CD4–CD8+CD27+CD45RA–CD45RO+
28	CD8+ effector memory T cells	CD45+CD66b–CD19–CD3+CD4–CD8+CD27–CD45RA–CD45RO+
29	CD8+ terminal effector T cells	CD45+CD66b–CD19–CD3+CD4–CD8+CD27–CD45RO–CD45RA+
30	Activated CD8+ T cells	CD45+CD66b–CD19–CD3+CD4–CD8+CD38+HLA-DR+
31	Double-negative T cells	CD45+CD66b–CD19–CD3+CD8–CD4–
32	CD4– MAIT/NKT cells	CD45+CD66b–CD19–CD3+CD4–CD28+CD161hi

Supplementary Table 2: Changes in population frequency of 32 immune cell populations

Population frequencies of the 32 immune cell types in stabilized/frozen samples. The frequencies were calculated from the last step of the cleanup gates [Cleanup_DNA2 (193Ir)], which represents live single cells, for all 32 immune cell populations. Frequencies are shown as change in population frequency calculated as follows:

Delta population frequency (%): frequency of fixed sample – frequency of freshly stained sample

	Stain-Preserve Workflow (PROT1)			Stain-Preserve Workflow (Cytodelics)			Preserve-Stain Workflow (PROT1)			Preserve-Stain Workflow (Cytodelics)		
	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3	Donor 1	Donor 2	Donor 3
CD66b+	–6.22	–3.21	–9.19	–3.07	–1.71	–1.94	–5.89	–4.83	–6.51	–0.45	0.51	–0.8
Neutrophils	–6.36	–3.18	–9.2	–3.37	–1.64	–2.05	–6.16	–4.89	–6.6	–0.58	0.56	–0.78
Eosinophils	0.04	–0.02	0	0.07	–0.07	–0.01	–0.03	–0.14	–0.01	0.05	–0.04	0.02
CD45+ (TBMNK)	5.43	2.65	8.11	2.1	0.81	0.29	5.79	4.37	5.84	0.51	–0.59	0.76
Basophils	0.06	0.04	0.29	–0.13	0.01	0.03	0.16	0.03	0.3	–0.08	–0.02	0.18
Dendritic cells (DCs)	0	0.06	0.26	0	–0.05	0.23	–0.33	–0.07	0.58	–0.16	–0.15	0.35
Plasmacytoid DCs	–0.03	–0.03	0.01	0.02	–0.01	0.04	–0.15	–0.1	0.03	–0.02	–0.02	0.01
CD38+ cDCs	0.02	0	0.03	0.02	0	0.03	–0.15	–0.13	0.06	–0.11	–0.13	0.07
Total monocytes	0.43	0.37	1.94	0.47	–0.01	1.72	–1.29	–1.82	1.67	–3.07	–4.06	–2.84
Classical monocytes	0.3	0.27	1.45	0.28	–0.11	1.14	–1.22	–1.65	1.11	–2.84	–3.75	–2.72
Transitional monocytes	0.07	–0.05	0.35	0.2	0.18	0.47	0	–0.16	0.07	–0.08	–0.19	–0.05
Nonclassical monocytes	0.03	0.05	0.2	0.04	0.01	0.23	0.02	–0.06	0.58	0	0.02	0.16
Natural killer cells	–0.04	0.18	0.09	–0.29	0.12	–0.15	0.03	0.36	0.4	–0.3	0.23	0.25
Total B cells	0.65	0.38	1.44	0.1	0.1	0.11	0.44	0.55	0.64	–0.39	–1.23	0.07
Naive B cells	0.51	0.37	1.21	0.05	0.07	0.05	0.44	0.68	0.38	–0.15	–0.79	0.02
Memory B cells	0.14	0	0.23	0.05	0.03	0.06	0	–0.14	0.26	–0.24	–0.44	0.04
Plasmablasts	–0.01	–0.02	0.01	0.01	–0.04	0.02	–0.02	–0.03	0.02	–0.02	–0.04	0.01
Total T cells	4.41	1.99	4.4	2	0.78	–1.62	3.95	2.93	1.55	0.85	0.55	–1.9
CD4+ T cells	3.33	0.91	2.58	2.02	0.37	0.16	2.84	1.21	0.84	0.89	0.41	–0.23
CD4+ naive T cells	2.01	0.58	0.97	1.55	0.4	0.43	2.24	1.4	0.69	0.59	0.41	0.18
CD4+ central memory T cells	0.99	0.27	1.24	0.5	0.13	0.84	0.4	–0.3	0.11	0.28	–0.07	–0.21
CD4+ effector memory T cells	0.13	–0.01	0.44	–0.13	–0.17	–1.03	–0.06	0.03	–0.3	–0.07	0.04	–0.45
CD4+ terminal effector T cells	–0.01	0.01	–0.08	–0.02	0.01	–0.1	0.19	0.04	0.25	0.1	0.02	0.24
Activated CD4+ T cells	0.07	–0.03	–0.01	0.06	0.06	0.16	–0.1	–0.23	–0.6	–0.16	–0.27	–0.65
CD8+ T cells	0.64	0.44	1.77	0.06	0.6	–1.29	0.07	–0.54	–0.29	–0.34	–0.32	–1.92
CD8+ naive T cells	0.41	0.56	0.86	0.41	0.8	0.34	0.52	1.26	0.58	0.09	0.54	–0.24
CD8+ central memory T cells	0.15	–0.16	0.5	–0.01	–0.05	0.08	–0.57	–1.01	–0.45	–0.33	–0.49	–0.59
CD8+ effector memory T cells	0.09	0.08	0.07	–0.11	–0.01	–0.27	–0.21	–0.18	–0.23	–0.18	–0.06	–0.2
CD8+ terminal effector T cells	–0.05	–0.12	0.32	–0.21	–0.08	–1.41	0.18	–0.57	–0.15	0.05	–0.27	–0.85
Activated CD8+ T cells	–0.03	–0.29	–0.15	0.05	0.12	0.01	–0.11	–0.82	–1.21	–0.15	–0.91	–1.31
Double negative T cells	0.43	0.61	0	–0.1	–0.14	–0.73	0.99	2.29	1.04	0.32	0.52	0.4
CD4– MAIT/NKT cells	0.01	–0.24	0.28	0.06	–0.17	0.2	–0.28	–0.89	–0.34	–0.23	–0.51	–0.21

Supplementary Table 3: Immune checkpoint markers (PD-1 and PD-L1) for customization of 20-marker panel

Metal-conjugated PD-1 and PD-L1 antibodies available from Standard BioTools for the customization of the Human Broad Immune Profiling CyTOF Panel, 20 Antibodies. For a full list of immune checkpoint markers, visit store.standardbio.com.

Marker	Clone	Metal	Part Number
CD279/PD-1	EH12.2H7	174Yb	3174020
CD279/PD-1	EH12.2H7	175Lu	3175008
CD274/PD-L1	29E.2A3	148Nd	3148017
CD274/PD-L1	29E.2A3	159Tb	3159029
CD274/PD-L1	29E.2A3	175Lu	3175017
CD274/PD-L1	MIH1	169Tm	3169029

References

1. Impact of Cryopreservation on Performance of the Maxpar Direct Immune Profiling System Application Note (FLDM-00089)
2. The Benefits of Palladium Barcoding on Data Quality and Workflow Application Note (FLDM-00012)
3. Approach to Bivariate Analysis of Data Acquired Using the Maxpar Direct Immune Profiling Assay Technical Note (400248)
4. Stewart, J.C. et al. "Changes in fluorescence intensity of selected leukocyte surface markers following fixation." *Cytometry Part A* 71 (2007): 379–385.
5. Rybakowska, P. et al. "Stabilization of human whole blood samples for multicenter and retrospective immunophenotyping studies." *Cytometry Part A* 99 (2021): 524–537.
6. Schulz, A.R. et al. "Stabilizing antibody cocktails for mass cytometry." *Cytometry Part A* 95 (2019): 910–916.

Learn more at **standardbio.com**

Or contact: tech.support@standardbio.com

CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000
South San Francisco, CA 94080 US
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
standardbio.com

SALES
North America | +1 650 266 6000 | info-us@standardbio.com
Europe/Middle East/Africa/Russia | +33 1 60 92 42 40 | info-europe@standardbio.com
Latin America | +1 650 266 6000 | info-latinamerica@standardbio.com
Japan | +81 3 3662 2150 | info-japan@standardbio.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | info-china@standardbio.com
All other Asia-Pacific countries/India/Australia | +1 650 266 6000 | info-asia@standardbio.com

FLDM-01322 Rev 03 072025
20-Marker Core Immune Flow Cytometry Panel App Note