Compatibility of new platinum-conjugated antibodies with standard CyTOF workflows

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Introduction

Metals conjugated by CyTOF® technology is a powerful platform for high parameter multiplex single-cell analysis. 2 Pt-labeled antibodies demonstrate novel applications in multiple workflows and high yielding mass spectrometers to allow simultaneous detection of multiple isotypes in complex biological samples. The catalog of precious metals (Pd-labeled and CD45 antibodies) has further expanded in the multiplicity of mass spectrometry by providing additional channels for antibody detection.

In this study, we introduce 2 platinum channels, which were previously used for viability staining and bulk cell identification, for antibodies detecting staining and human CD45 (HECD1) antibodies with multispecific rabbit polyclonal (Rh. anti-CD45; Rh. anti-CD45) superparamagnetic glycoprotein. This work demonstrates the capability of these novel platinum-labeled and CD45 antibodies (Pt CD45) for staining human peripheral blood mononuclear cells (PBMC) in suspension assays and validates that, comparability of existing Maxpar cell staining reagents, Cytoplasm/Secreted antibodies and CD45 stabilization and labeling reagents. In addition, we demonstrate compatibility of these novel Pt CD45 antibodies with Maxpar Intracellular staining protocols, indicating the potential of CyTOF® technology for expanded panel designs and workflows.

Materials and methods

Pt-CD45 antibodies in palladium barcoding

- **Pt-CD45 antibody was selected as a representative Pt labeled antibody. PT barcoding was performed following the protocol described in the Cytosoft 20 Phs Pd Barcoding Kit (Fluidigm)**.
- The experimental workflow is shown in Figure 3. The PBMC samples were stained with either Cell Fix/Perm S buffer followed by surface staining (A) or Cell Perm S buffer followed by surface staining (B). Results of the surface staining process were then analyzed on the CyTOF® platform.

Pt-CD45 antibodies in whole blood staining

- **Pt-CD45 was used as an alternative Pt labeled antibody. The staining procedure is shown in Figure 4. **
- **The Pt-CD45 antibody was confirmed for use in whole blood staining using Maxpar buffers for cytokines or nuclear antigen staining.**

Pt-labeled antibodies in intracellular staining

- **Intercellular staining was performed using the Maxpar intracellular staining protocols (FLDM 400278), Maxpar, and Pt-CD45 antibodies in the Pt-CD45 antibody.**
- **The experimental workflow is shown in Figure 5. The PBMC samples were stained with CD95 monoclonal antibodies (CYTOMX-002) as a viability indicator and labeled. Anti-CD45 antibody was not required for nuclear antigen staining.**
- **The procedure of Maxpar Intracellular staining protocols was first diluted to 1 μL per 100 μL test.**
- **Anti-CD45 antibodies were used for viability staining.**

Pt-CD45 antibodies in cell surface staining

- **Cell staining was performed following the Maxpar Cell Surface Staining with Fix and Perm (FLDM-00225).**
- **Packed cell volume (PCV) was used as a control, while Maxpar stain (FLDM-00225) was used to detect Cytoplasm/Secreted antibodies.**

Pt labeled antibodies are compatible with palladium barcoding protocol

- **Comparing the functional performance of the Pt-labeled antibodies with those of control antibody (Rh. anti-CD45) or control antibody (Rh. anti-CD45) was used to stain whole blood samples from a healthy donor. Specificity of Pt-CD45 antibody was assessed by comparing the functional performance of Rh anti-CD45 antibody with Pt-CD45 antibody.**

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Conclusions

- **Pt-CD45 labeled antibodies can be used in the same barcoding protocol as the control antibody (Rh anti-CD45) in suspension or surface staining.**
- **This new Pt-labeled antibody showed compatibility as a surface marker used in combination with intracellular staining processes and palladium barcoding protocol.**
- **This new Pt-labeled antibody can be used in cell surface samples to provide comparable functional performance for both Pt-CD45 antibody and do not result in any compromised staining other cell function and permeabilization.**

- **Overall, we demonstrated the utility and versatility of the Pt-labeled antibodies in suspension or surface staining applications.**

References

2. Fluidigm Technical Note: Using Multiplexed Palladium Concluding Maxpar in Suspension Mass Cytometry, FLDM-00228
3. Fluidigm Application Note: Staining CD45 and Intracellular Antibody Staining with Fix and Perm, FLDM-00225
4. Fluidigm Application Note: Using Multiplexed Palladium Concluding Maxpar in Suspension Mass Cytometry, FLDM-00228

Acknowledgments and ethics statement

We are grateful to the Canadian Blood Services and the Blood donors who made this research possible. Donors provided IRB approved consent. The views expressed herein do not necessarily represent the views of Canadian Blood Services or the federal, provincial or territorial governments of Canada.