Platinum-Labeled CD45 Antibodies

Frequently Asked Questions

Samples have high background levels in ^{194}Pt. Is this normal?

The abundance sensitivity signal from ^{193}Ir channel can spill over into the platinum 194 (^{194}Pt) channel. Cell-ID™ Intercalator-Ir (201192A/B) should be titrated so that the signal intensity of the ^{191}Ir channel lies in the optimal range of 300–1,000 dual counts.

How can metal impurity of Pt-conjugated antibodies be determined?

Signal overlap contributed by isotopic impurity, abundance sensitivity, and oxides, if any, is calculated within Maxpar® Panel Designer v2.0 for each antibody in the panel, including antibodies conjugated to Pt.

For a matrix showing purity for each Pt isotope, see the technical bulletin Purity Matrix for Cadmium and Platinum (FLDM-00038) on the Fluidigm website or ask your local field applications specialist.

Are there special considerations when using Pt-conjugated antibodies?

Yes. Here are some considerations for use:

- Cell-ID Intercalator-Ir (201192A/B) should be titrated for use when staining with ^{194}Pt-labeled antibodies.
- Viability staining should be performed with either Cell-ID Intercalator-^{103}Rh (201103A/B) or monoisotopic Cell-ID Cisplatin reagents (201194, 201195, 201196, 201198) in a different mass channel to avoid direct mass overlap.
- If you are using custom Pt-conjugated antibodies, review the anticipated signal overlap values in Maxpar Panel Designer v2.0.
- Among the 4 Pt isotopes, ^{194}Pt- and ^{195}Pt-conjugated antibodies produce the least signal overlap into other Pt channels, and ^{198}Pt produces the most. The ^{198}Pt signal overlap remains manageable when designing panels that use multiple Pt reagents together.

For more information, see the technical note Using Monoisotopic Platinum-Containing Reagents for Suspension Mass Cytometry (FLDM-00446).

Can Pt-CD45 antibodies be used on whole blood?

Yes. Pt-CD45 can be used to stain fresh or red blood cell lysed whole blood. Pt-CD45 antibodies should be titrated on comparable whole blood samples. Signal intensities of Pt-CD45 may differ in whole blood relative to peripheral blood mononuclear cells (PBMC).

Special considerations must be taken with whole blood, as the granulocyte population has a lower CD45 expression than lymphocytes and myeloid cells. For CD45 antibody-based live-cell barcoding on whole blood or PBMC, combinations of 3 CD45 antibodies (CD45-barcodes) must be titrated on their respective sample types to determine the optimal concentration. When using samples from various disease states or when stimulating samples that might have lower CD45 expression, perform pilot experiments to assess barcode separation.

Are Pt-CD45 antibodies compatible with the Maxpar Direct Immune Profiling Assay?

Pt-CD45 antibodies are compatible for use with the Maxpar® Direct™ Immune Profiling Assay™ using healthy PBMC and whole blood. For special considerations when using Pt-CD45 antibodies on whole blood samples, see the previous question.

It is important to note that ^{89}Y-CD45 (HI30 clone) is included in the dry antibody pellet, and therefore epitope competition must be considered when titrating Pt-CD45 antibodies for live-cell barcoding in conjunction with the Maxpar Direct Immune Profiling Assay.

For more information, see the CYTO® 2021 Fluidigm poster Cohen et al., Expanding live-cell barcoding applications with mass cytometry using cadmium- and platinum-labeled antibodies.

Are Pt-CD45 antibodies compatible with Maxpar cell staining protocols?

Yes. Pt-CD45 antibodies are compatible for use with the cell surface staining protocol Maxpar Cell Surface Staining with Fresh Fix (400276) and the intracellular staining protocols Maxpar Nuclear Antigen Staining with Fresh Fix (400277), Maxpar Phosphoprotein Staining with Fresh Fix (400278), and Maxpar Cytoplasmic/Secreted Antigen Staining with Fresh Fix (400279).

For more information, see CYTO 2021 Fluidigm posters Cohen et al., Expanding live-cell barcoding applications with mass cytometry using cadmium- and platinum-labeled antibodies; and Yao et al., Compatibility of new platinum-conjugated antibodies with standard CyTOF® workflows.

Can Pt-CD45 antibodies be used on fixed cells?

Yes. Pt-CD45 (clone HI30) can be used to stain fixed PBMC and provides similar functional performance and signal intensities as staining unfixed cells.

Are individually used Pt-CD45 antibodies compatible with the Cell-ID 20-Plex Pd Barcoding Kit?

Pt-CD45 antibodies are compatible with Cell-ID 20-Plex Pd Barcoding Kit (201060).

Can I use Pt-conjugated antibodies with Cell-ID Cisplatin reagents?

Yes, monoisotopic Cell-ID Cisplatin (201194, 201195, 201196, 201198) and Pt-conjugated antibodies with different Pt isotopes can be used together. However, monoisotopic Pt reagents are not compatible for use with natural-abundance Cell-ID Cisplatin (201064) due to direct mass overlap.

For more information, see the technical note Using Monoisotopic Platinum-Containing Reagents for Suspension Mass Cytometry (FLDM-00446).

Can I use Pt-CD45 antibodies for live-cell barcoding with other Maxpar CD45 antibodies?

Yes. For more information, see the application note Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry (FLDM-00488).

Can I debarcode a file that uses Pt-CD45 antibodies for live-cell barcoding with the CyTOF Software Debarcoder?

Yes. The CyTOF Software Debarcoder can debarcode any 6-choose-3 scheme using metals between 101 and 209 Da. For 7-choose-3 or other barcoding schemes and inclusion of ^{89}Y-CD45, there are alternate debarcoding programs. For assistance, contact your local field applications specialist.

Are Pt-CD45 antibodies compatible with Cell-ID Intercalator-^{103}Rh for live/dead discrimination?

Yes. Cell-ID Intercalator-^{103}Rh as a viability stain is compatible for use with Pt-CD45 antibodies. Using Cell-ID Intercalator-^{103}Rh instead of Cell-ID Cisplatin for viability staining enables the alternate use of the platinum channels.

How do signal intensities of Pt-CD45 antibodies compare to those of other Maxpar CD45 antibodies?

Signal intensities of Pt-CD45 antibodies are comparable to those of yttrium (Y)- and cadmium (Cd)-conjugated CD45 antibodies and slightly lower than lanthanide-conjugated CD45 antibodies.

For a comparison of Pt-CD45 with ^{89}Y-CD45, see the technical note Using Monoisotopic Platinum-Containing Reagents for Suspension Mass Cytometry (FLDM-00446). For mixed-metal CD45 barcodes containing Cd, Pt, and Y together, see the application note Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry (FLDM-00488).

What is the best monoisotopic Cell-ID Cisplatin to use if I would like to use 3 of the 4 Pt-CD45 antibodies for live-cell barcoding?

All combinations of 3 Pt-CD45 antibodies (Pt-CD45 barcode) are compatible for use with monoisotopic Cell-ID Cisplatin in the fourth Pt channel for viability staining. The Pt-CD45 barcode that produced the least signal overlap into the fourth platinum channel was 194Pt-195Pt-196Pt, and the Pt-CD45 barcode that produced the most signal overlap was 194Pt-196Pt-198Pt. It is essential to titrate Pt-CD45 barcodes and Cell-ID Intercalator-Ir to minimize signal overlap.

For more information, see the technical note Using Monoisotopic Platinum-Containing Reagents for Suspension Mass Cytometry (FLDM-00446).

How long are Pt-CD45 antibodies stable?

The Pt-CD45 antibodies should be used within 6 months upon receipt.

Can I order an antibody custom-conjugated to Pt?

Custom antibody conjugations to Pt are available through our Maxpar Antibody Conjugation Service for Mass Cytometry.

For technical support visit techsupport.fluidigm.com. | For general support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | support.northamerica@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com
China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com **Europe/Middle East/Africa/Russia** +33 1 60 92 42 40 | eu.support@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com