

# Platinum (Pt)-Labeled CD45 Antibodies

## Frequently Asked Questions

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

The abundance sensitivity signal from  $^{193}\text{Ir}$  channel can spill over into the  $^{194}\text{Pt}$  channel. Cell-ID™ Intercalator-Ir (201192A/B) should be titrated so that the signal intensity of the  $^{191}\text{Ir}$  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  $^{194}\text{Pt}$ -labeled antibodies.
- Viability staining should be performed with either Cell-ID Intercalator- $^{103}\text{Rh}$  (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,  $^{194}\text{Pt}$ - and  $^{195}\text{Pt}$ -conjugated antibodies produce the least signal overlap into other Pt channels, and  $^{198}\text{Pt}$  produces the most. The  $^{198}\text{Pt}$  signal overlap remains manageable when designing panels that use multiple Pt reagents together.

See Technical Note: Using Monoisotopic Cisplatin-Containing Reagents for Suspension Mass Cytometry (FLDM-00446) for more information.

## **Are Pt 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 \(400279\)](#).

## **Are individually used Pt antibodies compatible with the Cell-ID 20-Plex Pd Barcoding Kit or staining fixed cells?**

Pt-CD45 antibodies are compatible with Cell-ID 20-Plex Pd Barcoding Kit (201060). Additionally, Pt-CD45 antibodies can be used to stain fixed cells.

## **Can I use Pt 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.

See Technical Note: [Using Monoisotopic Cisplatin-Containing Reagents for Suspension Mass Cytometry \(FLDM-00446\)](#) for more information.

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

Yes. See Application Note: [Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry \(FLDM-00488\)](#) for more information.

## **Can I debarcode a file that uses Pt 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 <sup>89</sup>Y-CD45, there are alternate debarcoding programs. For assistance, contact your local field applications specialist.

## **Are Pt antibodies compatible with Cell-ID Intercalator-<sup>103</sup>Rh for live/dead discrimination?**

Yes. Cell-ID Intercalator-<sup>103</sup>Rh as a viability stain is compatible for use with Pt-CD45 antibodies. Using Cell-ID Intercalator-<sup>103</sup>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 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 <sup>89</sup>Y-CD45, see Technical Note: Using Monoisotopic Cisplatin-Containing Reagents for Suspension Mass Cytometry (FLDM-00446). For mixed-metal CD45 barcodes containing Cd, Pt, and Y together, see Application Note: Enabling Live-Cell Barcoding with Anti-CD45 Antibodies in Suspension Mass Cytometry (FLDM-00488).

## Can Pt antibodies be used on fixed cells?

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

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

All combinations of 3 Pt-CD45 antibodies are compatible for use with monoisotopic Cell-ID Cisplatin in the fourth Pt channel for viability staining.

See Technical Note: Using Monoisotopic Cisplatin-Containing Reagents for Suspension Mass Cytometry (FLDM-00446) for more information.

## Can Pt 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 PBMC.

## 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 will be available shortly through our Maxpar Antibody Conjugation Service for Mass Cytometry.

**For technical support visit [techsupport.fluidigm.com](https://techsupport.fluidigm.com). | For general support visit [fluidigm.com/support](https://fluidigm.com/support).**

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