Cell-ID Cisplatin-195Pt

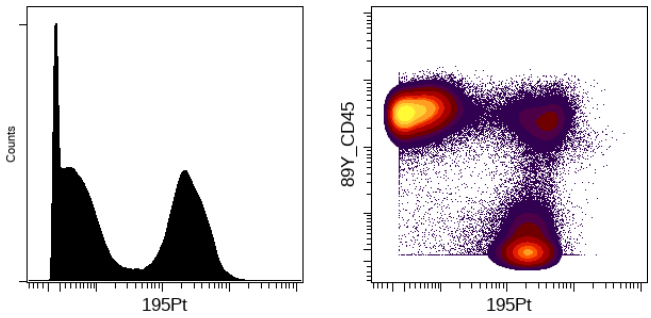
Catalog number, package size: 201195, 100 µL
Concentration: 1 mM

Storage: Upon receiving this product, aliquot and freeze at −20 °C. Frozen aliquots should be used only once after thawing.

Application: Cell viability on CyTOF® suspension mass cytometry systems

Technical Information

Description: Cell-ID™ Cisplatin-195Pt is a monoisotopic preparation of cisplatin containing the 195Pt isotope. Cisplatin is a stain that binds covalently to cellular proteins and labels cells with compromised cell membranes to a greater extent than live cells. Cell-ID Cisplatin-195Pt therefore specifically identifies dead cells if cells are incubated prior to fixation, or it identifies total cells if cells are incubated after cell fixation and permeabilization. Because cisplatin binds covalently to protein, cisplatin labeling is resistant to subsequent fixation, permeabilization, and washing steps used in intracellular staining protocols for mass cytometry.



Human PBMC were heat-killed by incubating at 55 °C for 1 hr and then added to live human PBMC stained with anti-CD45 (HI30)-89Y, to distinguish heat-killed (CD45−) and live cells (CD45+). The PBMC mixture was stained with Cell-ID Cisplatin-195Pt for 5 min. Singlet PBMC events were gated. The histogram (left) and the biaxial plot (right) display Cisplatin-195Pt and CD45 expression vs. 195Pt, respectively.

Important Product Notes

- Do not use Cell-ID Cisplatin-195Pt with natural abundance Cell-ID Cisplatin (201064) or platinum (195Pt)-labeled antibodies due to direct mass overlap.
- Upon receiving this product, divide into single-use aliquots and freeze them at −20 °C. Frozen aliquots of Cell-ID Cisplatin-195Pt should be used only once immediately after thawing to room temperature. Avoid multiple freeze/thaw cycles as this may alter the chemical and cell-binding properties of the reagent.
- We recommend that you determine the optimal staining concentration for Cell-ID Cisplatin-195Pt by titrating the reagent at concentrations between 0.2 and 1 µM for 5 min. Cisplatin staining intensity has been observed to increase with cell size (for example, cisplatin staining intensity for monocyte populations is greater than for lymphocyte populations). For optimal results with viability staining, titrate using media and cells that you will use in future experiments.
- Cell-ID Cisplatin-195Pt staining must be quenched with a solution that contains protein, such as Maxpar® Cell Staining Buffer (201068).
- Cell-ID Cisplatin-195Pt can be used for total cell staining if cells are incubated after cell fixation and permeabilization. For more information on this protocol, contact your local Field Applications Specialist.
- We strongly recommend that you titrate Cell-ID Cisplatin-195Pt prior to use with other monoisotopic cisplatin-containing reagents. You can use the Maxpar Panel Designer tool to determine the metal impurity of this product.
- For phosphoprotein analysis, perform Cell-ID Cisplatin-195Pt viability staining before cell stimulation with pre-warmed serum-free medium and quench the cisplatin stain with serum-containing medium.
- Detect the Cell-ID Cisplatin-195Pt metal isotope in the 195Pt mass channel of the Fluidigm CyTOF suspension system you will use for sample acquisition. Add the Cell-ID Cisplatin-195Pt metal isotope to your acquisition template (.tem) prior to acquisition of samples. Refer to your CyTOF system user guide for information on how to add elements to the acquisition template and run samples using CyTOF Software.

Before You Begin

Before using this product, refer to the following instructions for more information:

- Remove a single-use aliquot of Cell-ID Cisplatin-195Pt from −20 °C storage and thaw it to room temperature immediately before use.
- If applicable, pre-warm serum-free and serum-containing complete cell culture medium at 37 °C before beginning the protocol below.

Viability Staining Protocol

1. Wash cells with Maxpar PBS (201058) or pre-warmed serum-free medium. Centrifuge cells at 300 x *g* for 5 min, carefully aspirate the supernatant, and gently pipet to mix. Resuspend cells to 20 million cells per mL in Maxpar PBS or pre-warmed serum-free medium.
2. Prepare a 2X working solution of the pre-titrated Cell-ID Cisplatin-195Pt by diluting the Cell-ID Cisplatin-195Pt stock in Maxpar PBS or pre-warmed serum-free medium. (For example, add 2 µL of 1 mM Cell-ID Cisplatin-195Pt stock to 998 µL of pre-warmed serum-free medium to create a 2 µM Cell-ID Cisplatin-195Pt working solution.)
3. Add an equal volume of 2X working solution of Cell-ID Cisplatin-195Pt to the cell suspension. (For example, if you are staining 20 million cells in 1 mL, add 1 mL of 2X working solution of Cell-ID Cisplatin-195Pt.)
4. Mix well and incubate for 5 min at room temperature, or at 37 °C for analysis of phosphoproteins.
5. Quench the cisplatin stain and wash the cells with Maxpar Cell Staining Buffer or pre-warmed serum-containing medium, using **at least 5x the volume** of the cell suspension. (For example, add 10 mL of Maxpar Cell Staining Buffer or serum-containing medium to 2 mL of a 20 million cell suspension.) Centrifuge cells at 300 x *g* for 5 min, carefully aspirate the supernatant, and gently pipet to mix. Repeat for a total of 2 wash steps.
6. (Optional) For analysis of phosphoproteins, place the cells back in culture conditions for 15–30 min of rest before stimulation.
7. Proceed with staining surface and/or intracellular antigens for analysis by CyTOF suspension mass cytometry. Search for the applicable Maxpar cell staining protocols available at fluidigm.com for more information.

Related Products

Other monoisotopic Cell-ID Cisplatin reagents include: Cell-ID Cisplatin-194Pt (201194), Cell-ID Cisplatin-196Pt (201196), Cell-ID Cisplatin-198Pt (201198).

References

Fienberg, H.G. et al. "A platinum-based covalent viability reagent for single-cell mass cytometry." Cytometry Part A 81 (2012): 467–75.

Hartmann, F.J. et al. "A universal live cell barcoding-platform for multiplexed human single cell analysis." Scientific Reports 8 (2018): 10,770.

Majonis, D. et al. "Curious results with palladium- and platinum-carrying polymers in mass cytometry bioassays and an unexpected application as a dead cell stain." Biomacromolecules 12 (2011): 3,997–4,010.

Mei, H.E. et al. "Platinum-conjugated antibodies for application in mass cytometry." Cytometry Part A 89 (2016): 292–300.

Wei, S.C. et al. "Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade." Cell 170 (2017): 1120–1133.e17.

Safety

Use standard laboratory safety protocols. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.