

Cell-ID Cisplatin-195Pt

Catalog number, package size: 201195, 100 µL

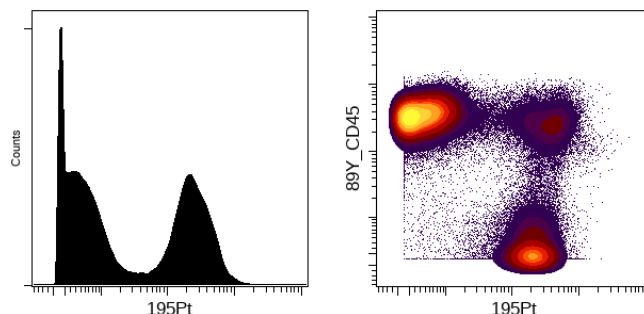
Concentration: 1 mM

Storage: Upon receiving this product, aliquot and freeze at $-20\text{ }^{\circ}\text{C}$. Frozen aliquots should be used only once after thawing.

Application: CyTOF® suspension mass cytometry

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, its labeling remains strong through subsequent cell handling steps used in downstream mass cytometry cell staining protocols.



Human PBMC were heat-killed by incubating at $55\text{ }^{\circ}\text{C}$ for 1 hr and then added to human PBMC stained with anti-CD45 (HI30)-89Y. The PBMC mixture was stained with Cell-ID Cisplatin-195Pt for 5 min and then gated as singlets as shown in the histogram (left) and the biaxial plot (right) of CD45 by 195Pt. The heat-killed cells appear as CD45⁻ since they were not stained with CD45.

Important Product Notes

- Upon receiving this product, divide into single-use aliquots and freeze them at $-20\text{ }^{\circ}\text{C}$. 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 cell staining solution that contains protein, such as Maxpar® Cell Staining Buffer (Cat. No. 201068).
- 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.

Viability Staining Protocol

- 1 Wash cells with Maxpar PBS (Cat. No. 201058) or serum-free medium. Centrifuge cells at $300 \times g$ for 5 min, carefully aspirate the supernatant, and gently pipet to mix.
- 2 Prepare a working solution of the pre-titrated Cell-ID Cisplatin-195Pt concentration by diluting the Cell-ID Cisplatin-195Pt stock in Maxpar PBS or serum-free medium. For example, add 1 µL of 1 mM Cell-ID Cisplatin-195Pt stock to 1 mL of Maxpar PBS or serum-free medium to create a 1 µM Cell-ID Cisplatin-195Pt working solution.
- 3 Resuspend cells to $1 \times 10^7/\text{mL}$ with the working solution of Cell-ID Cisplatin-195Pt.
- 4 Mix well and incubate at room temperature for 5 min.
- 5 Quench the cisplatin stain and wash the cells with serum-containing medium or Maxpar Cell Staining Buffer, using at least 5x the volume of the cell suspension. For example, add 5 mL of serum-containing medium or Maxpar Cell Staining Buffer to 1 mL of cell suspension. Centrifuge cells at $300 \times g$ for 5 min, carefully aspirate the supernatant, and gently pipet to mix. Repeat for a total of 2 wash steps.
- 6 Proceed with staining surface and/or intracellular antigens for analysis by CyTOF suspension mass cytometry.

Total Cell Staining Protocol

- 1 During the last 5 min of incubating cells with Cell-ID Intercalator-Ir (Cat. No. 201192) in Maxpar Fix and Perm Buffer (Cat. No. 201067), add pre-titrated Cell-ID Cisplatin-195Pt to a final recommended concentration of between 0.2 and 1 µM (1,000–5,000X dilution of 1 mM stock). For example, add 1 µL of Cell-ID Cisplatin-195Pt stock to 1 mL of cell suspension in Maxpar Fix and Perm Buffer to create a final 1 µM Cell-ID Cisplatin-195Pt solution.
- 2 Quench the cisplatin stain and wash the cells with Maxpar Cell Staining Buffer using at least 5x the volume of the cell suspension. For example, add 5 mL of Maxpar Cell Staining Buffer to 1 mL of 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.
- 3 Wash cells with preferred acquisition solution, such as Maxpar Water (Cat. No. 201069) or Maxpar Cell Acquisition Solution (Cat. No. 201240).
- 4 Proceed with preparing cells for sample acquisition by CyTOF suspension mass cytometry.

Related Products

Other monoisotopic cisplatin reagents include: Cell-ID Cisplatin-194Pt (Cat. No. 201194), Cell-ID Cisplatin-196Pt (Cat. No. 201196), Cell-ID Cisplatin-198Pt (Cat. No. 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.

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