Cell-ID Cisplatin

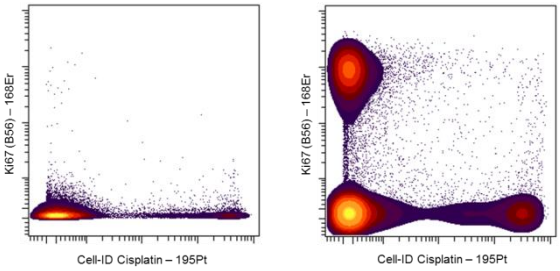
Catalog: 201064
Package size: 100 µL

Storage:
- Upon receiving this product, divide it into aliquots and freeze them at –20 °C.
- Frozen aliquots should be used only once after thawing.

WARNING Cell-ID™ Cisplatin is a mutagenic and carcinogenic agent. Before handling, refer to the safety data sheet (SDS) provided by the manufacturer and observe all relevant precautions.

Description

Cisplatin reacts with protein nucleophiles with which it can form covalent sulfhydryl bonds. This property makes it useful as a reagent to discriminate live cells from dead cells in mass cytometry. When cells are stained with Cell-ID Cisplatin, it rapidly enters dead cells with compromised cell membranes, where it nonspecifically labels total cellular protein to a greater extent than it does in live cells. Because cisplatin binds covalently to protein, cisplatin labeling is resistant to fixation, permeabilization, and washing steps used for intracellular staining for mass cytometry. Cell-ID Cisplatin is ideally detected in the 195Pt channel of the CyTOF® system.



Human PBMC were incubated for three days in media alone (left) or with PHA (right). Cells were then stained with Cell-ID Cisplatin, followed by fixation, permeabilization, and staining with 168Er anti-Ki-67 (B56). DNA+ cell singlet events are displayed in the analysis.

Important Product Notes

- Prolonged storage at room temperature and multiple freeze/thaws alter the chemical properties of Cell-ID Cisplatin, resulting in a reagent with increased potential for nonspecific binding, which could interfere with live/dead cell discrimination.
- Cell-ID Cisplatin staining for five minutes at a final concentration of 5 µM is suggested in the protocols below and has been found to work well for the majority of PBMC samples tested. However, these parameters should be optimized for individual cell types and experiments. We recommend staining with Cell-ID Cisplatin at a concentration between 1 and 5 µM for between 5 and 10 minutes.
- We recommend quenching Cell-ID Cisplatin staining with pre-warmed serum-containing medium. However, other cell staining solutions that contain protein, such as Maxpar® Cell Staining Buffer, may also be used.
- We have observed that cisplatin staining intensity increases with cell size. For example, cisplatin staining on monocyte populations is greater than staining on lymphocyte populations.

Before You Begin

- Remove a single-use aliquot of Cell-ID Cisplatin from –20 °C storage and thaw it to room temperature immediately before use.
- Pre-warm serum free and serum-containing complete cell culture medium at 37 °C before beginning the protocol below. Use the same media that are normally used for cell culture of your samples.
- Count cells to determine the cell viability of each sample. For best results, we recommend using samples with ≥80% cell viability.

Viability Staining Protocol Prior to Maxpar Cell Surface Staining

NOTE Cisplatin staining is done before surface antibody staining.

1. Dispense cells into individual 15 mL tubes for each sample.
2. Centrifuge each sample at 300 x *g* for 5 minutes, carefully aspirate the supernatant, and mix well by gently pipetting.
3. If cells were prepared in a serum-containing medium, wash cells to remove residual protein by adding 1 mL of pre-warmed serum-free medium. Centrifuge at 300 x *g* for 5 minutes, carefully aspirate the supernatant, and gently pipet to mix. If cells are in protein-free medium or buffer, then proceed to step 4.
4. Resuspend the cells to 2 x 10^7 cells/mL in pre-warmed serum-free medium.
5. Prepare a working solution of 10 µM cisplatin (2X concentration) by diluting the Cell-ID Cisplatin in pre-warmed serum--free medium (500X dilution from 5 mM stock). For example, add 2 µL of 5 mM stock to 1 mL of pre-warmed serum-free medium.
6. Add an equal volume of 10 µM cisplatin working solution to the cell suspension (final concentration of cisplatin is 5 µM). For example, if you are staining 1 mL of cells, add 1 mL of 10 µM cisplatin.
7. Mix well and incubate at room temperature for 5 minutes.
8. Quench the cisplatin staining by washing each sample with serum-containing medium or Maxpar Cell Staining Buffer, using 5x the volume of the stained cells. Centrifuge at 300 x *g* for 5 minutes, aspirate the supernatant, and gently pipet to resuspend the cell pellet.
9. Wash cells by adding 4 mL of Maxpar Cell Staining Buffer. Centrifuge at 300 x *g* for 5 minutes, aspirate supernatant, and gently pipet to mix.
10. Resuspend 1–3 million cells in 50 µL of Maxpar Cell Staining Buffer and proceed with the Maxpar Cell Surface Staining with Fresh Fix Protocol (PN 400276).
11. Detect Cell-ID Cisplatin in the 195Pt channel of the CyTOF system.

Viability Staining Protocol for Analysis of Phosphoproteins

NOTE Cisplatin staining is done before stimulation for phosphorylation.

1. Dispense cells into individual 15 mL tubes for each sample.
2. Centrifuge each sample at 300 x *g* for 5 minutes, carefully aspirate supernatant, and mix well by gently pipetting.
3. If cells were prepared in a serum-containing medium, wash cells to remove residual protein by adding 1 mL of pre-warmed serum-free medium. Centrifuge at 300 x *g* for 5 minutes, carefully aspirate the supernatant, and gently pipet to mix. If cells are in protein-free medium or buffer, then proceed to step 4.
4. Resuspend the cells to 2 x 10^7 cells/mL in pre-warmed serum-free medium.
5. Prepare a working solution of 10 µM cisplatin by diluting the Cell-ID Cisplatin in pre-warmed serum-free medium (500X dilution from 5 mM stock). For example, add 2 uL of 5 mM stock to 1 mL of pre-warmed serum-free medium.
6. Add an equal volume of 10 µM cisplatin working solution to the cell suspension (final concentration of cisplatin is 5 µM). For example, if you are staining 1 mL of cells, add 1 mL of 10 µM cisplatin.
7. Mix well and incubate at 37 °C for five minutes.
8. Quench the cisplatin staining by washing each sample with serum-containing medium, using 5x the volume of the stained cells. Centrifuge at 300 x *g* for 5 minutes, aspirate the supernatant, and gently pipet to mix.
9. Place the cells back in culture conditions for 15–30 minutes of rest before stimulation.
10. Proceed with the Maxpar Phosphoprotein Staining with Fresh Fix Protocol (PN 400278), including cell stimulation and fixation.
11. Detect Cell-ID Cisplatin in the 195Pt channel of the CyTOF system.

References

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.

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

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