

Cell-ID™ Cisplatin

Catalog #201064 (100 µL)



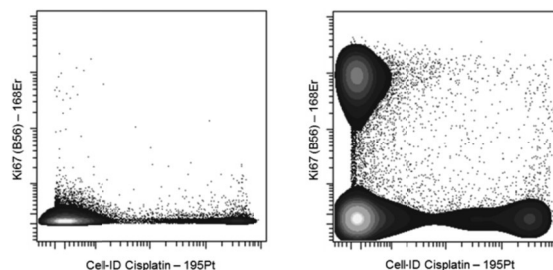
CHEMICAL HAZARD: Cell-ID Cisplatin is a mutagenic and carcinogenic agent. Before handling, refer to the Material Safety Data Sheet (MSDS), and observe all relevant precautions.

Storage:

Upon receiving this product it is necessary to aliquot and freeze at -20°C . Frozen aliquots should only be used once after thawing.

Description:

Cisplatin reacts with protein nucleophiles, with which it can form covalent sulfhydryl bonds, and it is this property that 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 non-specifically 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® mass cytometer.



Human PBMCs were incubated for 3 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-Ki67 (B56). DNA+ cell singlet events are displayed in the analysis.

Important Product Notes:

- Prolonged storage at room temperature and multiple freeze/thaws will alter the chemical properties of Cell-ID Cisplatin, resulting in a reagent with increased potential for non-specific binding, which could interfere with live/dead cell discrimination.
- Cell-ID Cisplatin staining for 5 minutes at a final concentration of $5\ \mu\text{M}$ is suggested in the protocol 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 $1\text{-}5\ \mu\text{M}$ for between 5-10 minutes.
- We recommended quenching Cell-ID Cisplatin staining with MaxPar® Cell Staining Buffer. However, other cell staining solutions which contain protein may also be used.

Viability Staining Protocol:

1. Wash cells with PBS, centrifuge at 300-400xg for 5 minutes and discard supernatant by aspiration.
2. Resuspend cells to $1 \times 10^7/\text{ml}$ in PBS and add Cell-ID™ Cisplatin to a final concentration of $5\ \mu\text{M}$ (1000X dilution of 5 mM stock solution, ie. $1\ \mu\text{L}$ Cell-ID Cisplatin added to 1 mL of cell suspension).
3. Mix well and incubate at room temperature for 5 minutes.
4. Quench staining with MaxPar® Cell Staining Buffer using 5X the volume of the cell suspension (ie. add 5 mL to 1 mL of cell suspension), centrifuge and discard supernatant by aspiration.
5. Proceed with usual procedure for staining surface or intracellular antigens for analysis by mass cytometry.
6. Detect Cell-ID Cisplatin in the 195Pt channel of the CyTOF mass cytometer.

Viability Staining Protocol for Analysis of Phospho-Proteins:

1. Wash cells with pre-warmed serum-free media, centrifuge at 300-400xg for 5 minutes and discard supernatant by aspiration.
2. Resuspend cells to 1×10^7 /ml in pre-warmed serum-free media and add Cell-ID™ Cisplatin to final concentration of 5 μ M (1000X dilution of 5 mM stock solution, ie. 1 μ L Cell-ID Cisplatin added to 1 mL of cell suspension).
3. Mix well and incubate at 37°C for 5 minutes.
4. Quench staining with pre-warmed complete serum-containing media using 5X the volume of the cell suspension (ie. add 5 mL to 1 mL of cell suspension), centrifuge and discard supernatant by aspiration.
5. Place cells back in culture conditions for 15-30 minutes to allow cells to “rest” prior to cell stimulation.
6. Proceed with usual phospho-protein staining procedure, including cell stimulation and fixation.
7. Detect Cell-ID Cisplatin in the 195Pt channel of the CyTOF mass cytometer.

References:

Curious results with palladium- and platinum-carrying polymers in mass cytometry bioassays and an unexpected application as a dead cell stain. Majonis D, Ornatsky O, Kinach R, Winnik MA. *Biomacromolecules*. 2011 Nov 14;12(11):3997-4010.

A platinum-based covalent viability reagent for single-cell mass cytometry. Fienberg HG, Simonds EF, Fantl WJ, Nolan GP, Bodenmiller B. *Cytometry A*. 2012 Jun;81(6):467-75.

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