

# Cell-ID Intercalator-<sup>103</sup>Rh—2000 μM

Catalog: 201103B  
Package size: 500 μL

## Storage:

- Upon receiving this product, divide it into aliquots and freeze them at -20 °C.
- Aliquots stored at 4 °C are stable for up to three months.
- Frozen aliquots should be used only once after thawing.



**WARNING** Before handling any chemicals, refer to the safety data sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

## Description

Cell-ID™ Intercalator-<sup>103</sup>Rh is a cationic nucleic acid intercalator that contains natural abundance rhodium (<sup>103</sup>Rh) and is used in CyTOF® system analysis for either discrimination of dead cells from live cells (if staining is done before cell fixation) or to discriminate single nucleated cells from doublets (if staining is done post-fixation). It is a live-cell membrane-impermeable dye.

## Important Product Notes

- Cell-ID Intercalator-<sup>103</sup>Rh—500 μM is a highly concentrated metal intercalator solution. It must be diluted in accordance with these protocols to avoid early failure of the detector.
- While dilutions of the 500 μM stock solution are suggested in the protocols below, the concentration can be titrated for individual cell types and experiments for optimal staining. It is suggested that the intercalator concentration in the staining solution not exceed 4 μM for cell singlet discrimination protocols. Cells with high mean values for intercalator-Rh in <sup>103</sup>Rh-versus-<sup>193</sup>Ir plots are considered dead, since the membrane-impermeable intercalator-Rh would have accumulated only in cells with a compromised membrane. These cells should be excluded in gating strategies.
- For live/dead staining of thawed cells including PBMC, rest thawed cells for at least one hour before proceeding with the addition of Cell-ID Intercalator-<sup>103</sup>Rh.
- Cell-ID Intercalator-<sup>103</sup>Rh staining diminishes with post-barcoding permeabilization protocols that use harsher permeabilization methods, such as methanol, for intracellular marker staining. To ensure discrete live/dead cell discrimination, Cell-ID Cisplatin (Cat. 201064) is recommended for experiments that require these harsher permeabilization methods.

## Live/Dead Staining Protocol

- 1 Add Cell-ID Intercalator-<sup>103</sup>Rh directly into cell culture flask at a dilution of 1:2,000 and return cells to incubator for 15 minutes.
- 2 (Optional) Count viable cells with trypan blue to confirm the percentage of viable cells observed with Cell-ID Intercalator-<sup>103</sup>Rh staining.
- 3 Proceed to stain cells as usual with Maxpar® metal-conjugated antibodies and, after fixation, with Cell-ID Intercalator-Ir (Cat. 201192A or 201192B) to identify dead cells.

## Cell Singlet Discrimination—Staining Protocol A

- 1 Before intercalating, fix cells.
  - If fixed with methanol, wash cells with PBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>) before proceeding.
  - If fixed with formaldehyde (3.7%, 30 min, RT) cells may be used directly.
- 2 Dilute Cell-ID Intercalator-<sup>103</sup>Rh 1:2,000 with PBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>).
- 3 Use 0.5 mL of working solution per 1×10<sup>6</sup> cells/tube.
- 4 Incubate 15–20 minutes at room temperature.
- 5 Wash cells with 2 mL PBS (without Ca<sup>2+</sup> or Mg<sup>2+</sup>) per tube. Repeat once.

## Cell Singlet Discrimination—Staining Protocol B

This protocol is for use with the Maxpar Cell Surface Staining Protocol (PRD012).

- 1 After cell staining is complete, prepare 1 mL of cell intercalation solution for each sample by diluting Cell-ID Intercalator-<sup>103</sup>Rh 1:4,000 into Maxpar Fix and Perm Buffer (Cat. 201067) and mix by vortexing.
- 2 Add 1 mL of the intercalation solution prepared in step 1 to each tube and gently vortex. Incubate for one hour at room temperature or leave overnight at 4 °C.

**Note:** Cells can be left at 4 °C in the intercalation solution up to 48 hours.

- 3 Wash cells by adding 2 mL of Maxpar Cell Staining Buffer (Cat. 201068). Centrifuge and discard supernatant by aspiration.
- 4 Repeat for a total of two washes with Maxpar Cell Staining Buffer.
- 5 Wash cells with 2 mL of Maxpar Water (Cat. 201069). Centrifuge and discard supernatant by aspiration.
- 6 Leave cells pelleted until ready to run on the CyTOF system. Immediately prior to data acquisition, adjust cell concentration to 2.5–5 × 10<sup>5</sup>/mL with Maxpar Water and filter cells into cell strainer cap tubes.
- 7 Acquire data on the CyTOF system.

**For technical support visit [www.fluidigm.com/support](http://www.fluidigm.com/support).**

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