Catalog #201103B (500 µL)

Cell-ID™ Intercalator-103Rh 2000 µM

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

NOTICE: HIGH CONCENTRATION. Cell-ID Intercalator-103Rh 2000 µM is a highly concentrated metal intercalator solution and must be diluted in accordance with this protocol to avoid early failure of the detector.

Description

Cell-ID Intercalator-103Rh is a cationic nucleic acid intercalator that contains natural abundance Rhodium (^{103}Rh) and is used in CyTOF® 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.

Note: While dilutions of the 2000 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 not to exceed 4 µM intercalator concentration in the staining solution for cell singlet discrimination protocols.

Live/Dead Staining Protocol

1. Add Cell-ID Intercalator-103Rh directly into cell culture flask at a dilution of 1:2000 and return cells to incubator for 15 minutes.
2. If desired count viable cells with Trypan Blue to confirm % viable observed with Cell-ID Intercalator-103Rh staining.
3. Proceed to stain cells as usual with MaxPar® metal-conjugated antibodies and, following fixation, with Cell-ID Intercalator-Ir (Fluidigm Cat. 201192A or 201192B) to identify dead cells.

Notes

- Cells with high Intercalator-Rh mean values in ^{103}Rh versus ^{193}Ir plot are considered dead since the membrane impermeable intercalator-Rh would have accumulated only in cells with a compromised membrane, and 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 addition of Intercalator-Rh in step 1.

Cell Singlet Discrimination—Staining Protocol A

1. Before intercalating, cells must be fixed.
   - If fixed with methanol, wash cells with PBS (without Ca^{2+} or Mg^{2+}) before proceeding.
   - Cells may be used directly if fixed with formaldehyde (3.7%, 30min, RT).
2. Dilute Cell-ID Intercalator-103Rh 1:2000 with PBS (without Ca^{2+} or Mg^{2+}).
3. Use 0.5 mL of working solution per $1x10^6$ cells/tube.
4. Incubate 15-20 mins at room temperature.
5. Wash cells with 2 mL PBS (without Ca^{2+} or Mg^{2+}) per tube. Repeat once.

Cell Singlet Discrimination—Staining Protocol B (for use with the MaxPar® Cell Surface Staining Protocol)

1. After cell staining is complete, prepare 1 ml of cell intercalation solution for each sample by diluting Cell-ID Intercalator-103Rh 1:4000 into MaxPar® Fix and Perm Buffer (Fluidigm 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 1 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 (Fluidigm 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 (Fluidigm Cat. 201069), centrifuge and discard supernatant by aspiration.
6. Leave cells pelleted until ready to run on CyTOF. Immediately prior to CyTOF data acquisition, adjust cell concentration to 2.5-5 x 10^5/ml with MaxPar Water and filter cells into cell strainer cap tubes.
7. Acquire data on CyTOF.

Technical Support

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