Catalog #201192B (500 µL)

Cell-ID™ Intercalator-Ir 500 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-Ir 500 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-Ir is a cationic nucleic acid intercalator that contains natural abundance Iridium (¹⁹¹Ir and ¹⁹³Ir) and is used for identifying nucleated cells in CyTOF® analysis. When cells are stained with Intercalator-Ir, it will bind to cellular nucleic acid, and detection of both stable isotopes will enable identification of nucleated cells. It is a live cell membrane-impermeable dye and therefore requires cells to be fixed and/or permeabilized before staining.

**Note:** 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 Cell-ID Intercalator staining. It is suggested not to exceed 1 µM intercalator concentration in the staining solution.

**Staining Protocol A**

1. Before intercalating, cells must be fixed.
   - If fixed with methanol, wash cells with PBS (without Ca²⁺ or Mg²⁺) before proceeding.
   - Cells may be used directly if fixed with formaldehyde (3.7%, 30min, RT).
2. Dilute Cell-ID Intercalator-Ir 1:2000 with PBS (without Ca²⁺ or Mg²⁺).
3. Use 0.5mL of working solution per 1x10⁶ cells/ tube.
4. Incubate 15-20 mins at room temperature.
5. Wash cells with 2 mL PBS (without Ca²⁺ or Mg²⁺) per tube. Repeat once.
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-Ir 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.