MaxPar® Intercalator-Ir 500 µM
Catalog #201192B (500 µL)

**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.** MaxPar 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:**
MaxPar Intercalator-Ir is a cationic nucleic acid intercalator that contains natural abundance Iridium ($^{191}$Ir and $^{193}$Ir) 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 MaxPar 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 Ca$^{2+}$ or Mg$^{2+}$) before proceeding
   - Cells may be used directly if fixed with formaldehyde (3.7%, 30min, RT)

2. Dilute MaxPar Intercalator-Ir 1:2000 with PBS (without Ca$^{2+}$ or Mg$^{2+}$).
3. Use 0.5mL of working solution per 1x10$^6$ cells/ tube.
4. Incubate 15-20 min at room temperature.
5. Wash cells with 2 mL PBS (without Ca$^{2+}$ or Mg$^{2+}$) 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 MaxPar Intercalator-Ir 1:4000 into MaxPar® Fix and Perm Buffer (DVS Sciences 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 (DVS Sciences 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 (DVS Sciences 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.