BioPAL *New ways to make measurements in the life sciences.*
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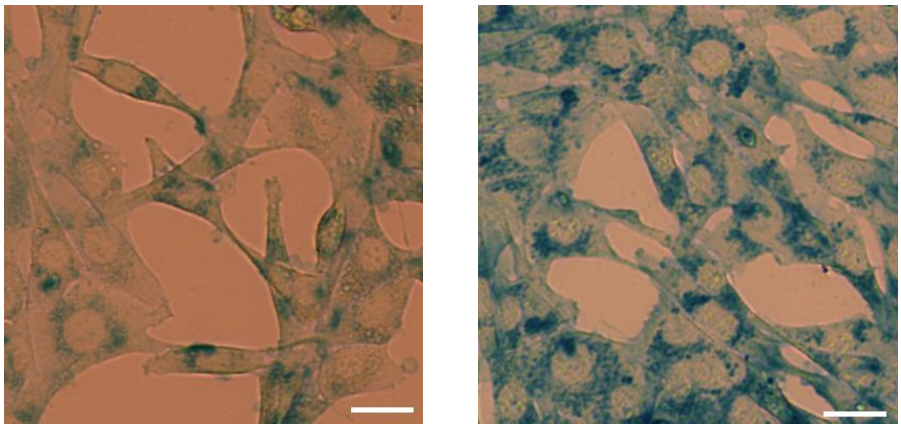
Prussian Blue Staining of Cells in Culture:

BioPAL's Prussian Blue Cell Staining Reagent Pack has been formulated for use with Molday ION and other iron based nanoparticles. The reagent pack is used to visualize the presence of iron deposits found inside cells after treatment with Molday ION.

Figure legend. Treatment of NIH3T3 cells with CL-30Q02-6 plus poly-L-lysine (Left Panel) or CL-30Q02-6A (Right Panel). Iron visualization was obtained using BioPAL's Prussian Blue Cell Staining Reagent Pack per the protocol described below. The degree of staining is subject to many factors including the type of contrast agent used, its concentration during cell labeling, and development time with BioPAL's Prussian Blue Cell Staining Reagent Pack. The bar represents 20 microns.



Materials:
Prussian Blue staining reagent pack consisting of Reagent A (50ml) and Reagent B (50ml) and Phosphate Buffered Saline (PBS) (100ml) .
Prussian Blue reagent pack may be purchased from BioPAL (Code CL-01-50)

Procedure:
1. **Cell fixation solution.** After cells have been fixed (see BioPAL Cell Fixation Procedure), prepare a sufficient amount of Prussian Blue cell staining reagent by mixing equal amounts of Reagent A with Reagent B for a **Working Solution**. Typically, 10 ml of Reagent A and 10 ml of Reagent B are mixed. Use the mixed reagents within 60 minutes. Aspirate any liquid covering fixed cells and add an amount of **Working Solution** sufficient to cover cells. Typically, we recommend a volume equal to the volume of medium that cells were grown in.

2. Allow the fixed cells and **Working Solution** to sit for 10 minutes at room temperature. During this time, if iron is present, a blue color will develop that can be seen without magnification. Aspirate the **Working Solution** from the cells and wash cells with PBS. Store the stained cells at 4°C in PBS to prevent dessication. The color is stable for 24 hours. Additional sensitivity may be obtained by allowing the development process to proceed for a longer time or by aspirating the **Working Solution** and applying the **Working Solution** a second time.