

Basic labeling protocol for nuclear dyes

This protocol provides general instructions for labeling the nuclei of cells using permeant or non-permeant nucleic acid dyes. The bulk of the content inside the nucleus is nucleic acid. For most nucleic acid stains the fluorescent signal is minimal before binding to nucleic acids, and there is a significant increase in fluorescence intensity after the dye has bound the nucleic acid.

The nuclear dye you use will most likely come with suggested working concentrations and incubation times given by the manufacturer. If your dye is supplied as a dried-down pellet or a stock in DMSO, you most likely will need to do serial dilutions, i.e., make a stock solution, and from that make a more dilute working solution that will be further diluted for a staining solution. Whew...that's a lot of dilutions! For most nuclear dyes, a 1 mM working solution that is diluted to a 1 μ M staining solution for your sample is a good place to start.


Staining medium can be a complete medium (i.e., your regular cell culture medium) or a saline-based buffer such as PBS or HBSS. Which you choose will depend on your experimental design. For example, you may choose to label in a complete medium if you are performing a viability assay to label the dead cells in a live cell population, or you may choose to label in a saline-based buffer if you are performing a counterstain during immunolabeling.


If you are optimizing for dye concentration, you will need to prepare a staining solution for each concentration you want to test. For example, if you want enough staining solution for 1 well in a 6-well plate, make 1 mL of staining solution for each concentration you will be using.


The volumes given in this protocol are good for a single well in a 6-well vessel or a single 35 mm vessel.


What you need


- Cells
- Staining medium
- Nuclear stain
- Fluorescence microscope with filter set matched to your fluorophore

1  Prepare 1 mL nuclear dye staining solution at your desired concentrations. If you are optimizing for dye concentration, you will need to prepare a staining solution for each concentration you want to test.

2  Remove medium from cells.

3  Add staining solution to cover the sample.

4  Incubate for 5–15 minutes at room temperature or 37°C for most nuclear dyes; some live-cell dyes may give you a better signal with longer incubation times.

5  Optional: For live-cell imaging, if you know your nuclear stain has a high binding affinity, you can remove the staining solution and wash to improve the signal:background ratio.

6  Image cells.

For more information, go to [lifetechnologies.com/imagingbasics](https://www.lifetechnologies.com/imagingbasics)