F-actin staining protocol

This protocol provides general instructions for labeling F-actin in fixed and permeabilized cells. Actin is one of the most abundant proteins found in cells and can be labeled with a fluorophore very easily when the monomers form a certain type of microfilament, referred to as F-actin. When phalloidin, a molecule that binds F-actin, is conjugated to a fluorophore, it becomes a very useful tool for cell biologists who are using fluorescence microscopy to study their cells. Labeling F-actin can help show the overall shape and structure of the cell and provide context for other fluorescent labels.

- For fixed and permeabilized cells
- Can be used before or after immunolabeling
- 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 that have been fixed and permeabilized (See Fix, Perm, and Block protocol)
- Phosphate buffered saline (PBS)
- Phalloidin conjugated to fluorophore
- Fluorescence microscope with filter set matched to your fluorophore

1		Prepare 1 mL F-actin staining solution in PBS from conjugated fluorophore. Phalloidins are typically very water soluble and stain F-actin at 100–200 nM 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 PBS from fixed and permeabilized cells.
3		Add 1 mL of staining solution.
4		Incubate for 20 minutes at room temperature.
5		Remove dye solution.
6		Wash 3 times with PBS.
7		Image cells.
8		Optional: To preserve your sample for long-term storage, you can also mount your sample in mounting medium. Before you start your staining experiment, it's a good idea to check that your fluorophores are compatible with the mounting medium you want to use.

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