Cell Navigator™ F-Actin Labeling Kit *Green Fluorescence*

Catalog number: 22661
Unit size: 500 Tests

Component	Storage	Amount
Component A: iFluor™ 488-Phalloidin	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL)
Component B: Labeling Buffer	Freeze (<-15 °C), Minimize light exposure	50 mL

OVERVIEW

Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria and nuclei etc. The selective labeling of live cell compartments provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to label F-actins of fixed cells in green fluorescence. The kit uses a green fluorescent phalloidin conjugate that is selectively bound to F-actins. This green fluorescent phalloidin conjugate is a high-affinity probe for F-actins with much higher photostability than the fluorescein-phalloidin conjugates. Used at nanomolar concentrations, phallotoxins are convenient probes for labeling, identifying and quantitating F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. The labeling protocol is robust, requiring minimal hands-on time. The kit provides all the essential components with an optimized staining protocol.

AT A GLANCE

Protocol summary

1. Prepare samples (microplate wells)
2. Remove the liquid from the plate
3. Add 100 µL/well of iFluor™ 488-Phalloidin working solution
4. Stain the cells at RT for 15 to 60 minutes
5. Wash the cells
6. Examine the specimen under fluorescence microscope at Ex/Em = 490/520 nm (FITC filter set)

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	FITC filter
Emission:	FITC filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 10 µL of iFluor™ 488-Phalloidin (Component A) to 10 mL of Labeling Buffer (Component B) to make 1X iFluor™ 488-Phalloidin working solution. Protect from light.

Note Different cell types might be stained differently. The concentration of iFluor™ 488-Phalloidin working solution should be prepared accordingly.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Perform formaldehyde fixation. Incubate the cells with 3.0% – 4.0% formaldehyde in PBS at room temperature for 10 – 30 minutes.

   Note Avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

2. Rinse the fixed cells 2 – 3 times in PBS.
3. **Optional:** Add 0.1% Triton X-100 in PBS into fixed cells for 3 to 5 minutes to increase permeability. Rinse the cells 2 – 3 times in PBS.
4. Add 100 µL/well (96-well plate) of iFluor™ 488-Phalloidin working solution into the fixed cells.
5. Stain the cells at room temperature for 15 to 60 minutes.
6. Rinse cells gently with PBS 2 to 3 times to remove excess dye before plate sealing.
7. Image cells using a fluorescence microscope with FITC filter set (Ex/Em = 490/520 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

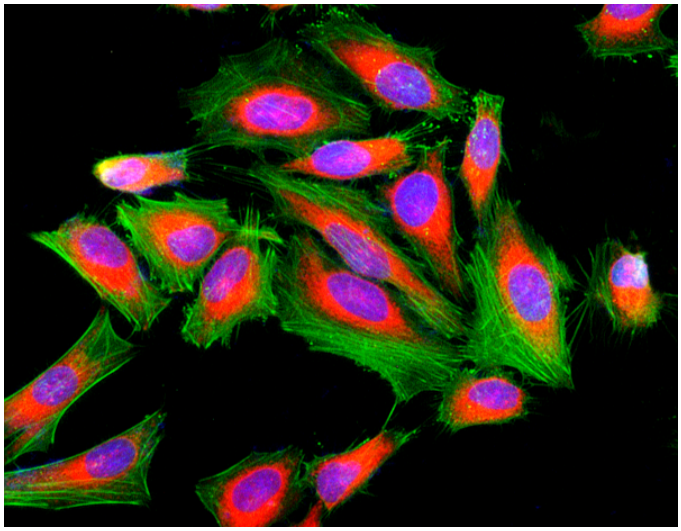

Figure 1.

Fluorescence image of HeLa cells fixed with 4% formaldehyde then stained with Cell Navigator™ F-Actin Labeling Kit *Green Fluorescence* in a Costar black 96-well plate. Cell were labeled with iFluor™ 488-Phalloidin (Cat#22261, Green) and nuclei stain DAPI (Cat#17507, Blue), respectively. Cell endoplasmic reticulum (ER) was stained with ER Red™ (Cat#22636, Red) before fixation.

DISCLAIMER