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

Catalog number: 22660
Unit size: 500 Tests

Component	Storage	Amount
Component A: iFluor™ 350-Phalloidin	Freeze (<-15 °C), Minimize light exposure	1 vial (50 uL)
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 blue fluorescence. The kit uses a blue fluorescent phalloidin conjugate that is selectively bound to F-actins. This blue fluorescent phalloidin conjugate is a high-affinity probe for F-actins. 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™ 350-Phalloidin working solution
4. Stain cells at RT for 15 to 60 minutes
5. Wash cells
6. Examine the specimen under microscope at Ex/Em = 350/450 nm

Important Warm all the components to room temperature before opening.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	350 nm
Emission:	450 nm
Instrument specification(s):	DAPI channel
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 10 µL of iFluor™ 350-Phalloidin (Component A) to 10 mL of Labeling Buffer (Component B).

Note Different cell types might be stained differently. The concentration of iFluor™ 350-Phalloidin working solution should be prepared accordingly. Protect from light.

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 1X iFluor™ 350-Phalloidin working solution into the fixed cells, and stain the cells at room temperature for 15 to 60 minutes.

5. Rinse cells gently with PBS 2 to 3 times to remove excess dye before plate sealing. Image by using the DAPI channel.

EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at: https://www.aatbio.com/products/cell-navigator-f-actin-labeling-kit-blue-fluorescence

DISCLAIMER