Cell Navigator™ F-Actin Labeling Kit

Blue Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 22660 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

Our Cell Navigator™ fluorescence labeling kits are a set of fluorescence imaging tools for labeling sub-cellular organelles such as membranes, lysosomes, mitochondria, 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 in fixed cells with 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. When 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 kit provides all the essential components with an optimized labeling protocol, which is robust, requiring minimal hands-on time. It is an excellent tool for preserving the fluorescent images of particular cells, and can also be used for fluorescence microscope demonstrations by using DAPI filter (Ex/Em = 350/450 nm).

Kit Components

Components	Amount
Component A: iFluor™350-Phalloidin	1 vial
Component B: Labeling Buffer	50 mL
Component C: Methanol	1 vial (1.5 mL)

Assay Protocol

Brief Summary

Prepare samples (microplate wells) → Remove the liquid from the plate → Add 100 µL/well of iFluor™ 350-Phalloidin solution → Stain the cells at RT for 15 to 60 min → Wash the cells → Examine the specimen under microscope at Ex/Em = 350/450 nm

Note: Warm all the components to room temperature before opening.

1. Prepare 50X iFluor™ 350-Phalloidin stock solution:

Add 1 mL of methanol (Component C) into the iFluor™ 350-Phalloidin vial (Component A) to make 50X stock solution.

Note: The unused iFluor™ 350-Phalloidin stock solution should be stored at -20 °C, and protected from lights.

2. Stain the cells:

2.1 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.

2.2 Rinse the fixed cells 2–3 times in PBS.

2.3 Optional: Add 0.1% Triton X-100 in PBS into fixed cells (from Step 2.2) for 3 to 5 minutes to increase permeability. Rinse the cells 2–3 times in PBS.

2.4 Prepare 1X iFluor™ 350-Phalloidin working solution by diluting 200 µL of 50X iFluor™ 350-Phalloidin stock solution (from Step 1.1) into 10 mL of Labeling Buffer (Component B).
2.5 Add 100 µL/well (96-well plate) of iFluor™ 350-Phalloidin working solution (from Step 2.4) into the fixed cells (from Step 2.2 or 2.3), and stain the cells at room temperature for 15 to 60 minutes.
2.6 Rinse cells gently with PBS 2 to 3 times to remove excess dye before plate sealing and imaging by using DAPI channel.

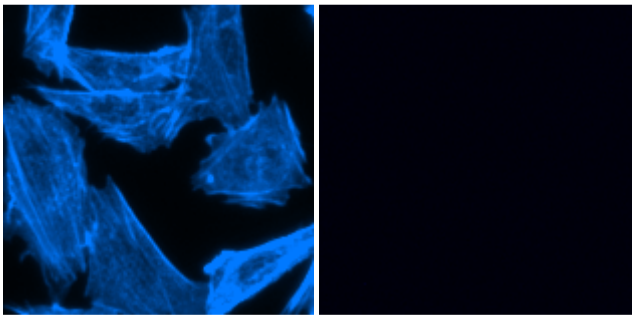

Figure 1. Images of CPA cells fixed with formaldehyde and stained with Cell Navigator™ F-Actin Labeling Kit *Blue Fluorescence* in a 96-well Costar black plate A: Label the cells with 1X iFluor™ 350-Phalloidin for 30 min only. B: Treat the cells with phalloidin for 10 min, then stain them with 1X iFluor™ 350-Phalloidin for 30 min.

References

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2. Johnson S C, Nancy M. McKenna M N, and Wang Y (1988). Association of microinjected myosin and its subfragments with myofibrils in living muscle cells. J Cell Biol, 107(6), 2213.
3. Wang K, Feramisco JR, Ash JF (1982). Fluorescent localization of contractile proteins in tissue culture cells. Methods Enzymol, 85 Pt B, 514.
4. Miki M, Barden JA, dos Remedios CG, Phillips L, Hambly BD (1987). Interaction of phalloidin with chemically modified actin. Eur J Biochem 165, 125.
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