Cell Navigator™ F-Actin Labeling Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22664 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

Actin is a globular, roughly 42-kDa protein found in almost all eukaryotic cells. It is also one of the most highly-conserved proteins, differing by no more than 20% in species as diverse as algae and humans. Actin is the monomeric subunit of two types of filaments in cells: microfilaments, one of the three major components of the cytoskeleton, and thin filaments, part of the contractile apparatus in muscle cells. Actin participates in many important cellular processes including muscle contraction, cell motility, cell division and cytokinesis, vesicle and organelle movement, cell signaling, as well as the establishment of cell junctions and cell shape.

Our Cell Navigator™ fluorescence imaging kits are a set of fluorescence 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 red fluorescence. The red fluorescent phalloidin conjugate, which is selectively bound to F-actins, is a high-affinity probe for F-actins. The red fluorescent phalloidin conjugate has Ex/Em = 594/610 nm, spectrally identical to the widely used Texas Red® conjugates. Used at nanomolar concentrations, phallotoxins can be conveniently used to label, identify and quantitate F-actins in formaldehyde-fixed and permeabilized tissue sections, cell cultures or cell-free experiments. The red fluorescent phalloidin conjugate has good thermal and photo stability. The kit provides all the essential components with an optimized labeling protocol. It is an excellent tool for preserving fluorescent images of particular cells, and can also be used for fluorescence microscope demonstrations using a Texas Red® filter (Texas Red is the trademark of InVitrogen).

Kit Components

Components	**Amount**
Component A: iFluor™ 594-Phalloidin	1 vial (50 µL)
Component B: Labeling Buffer	50 mL

Assay Protocol

Brief Summary

Prepare samples (microplate wells) → Remove the liquid from the plate→ Add 100 µL/well of iFluor™ 594-Phalloidin solution → Stain the cells at RT for 15 to 60 minutes → Wash the cells → Examine the specimen under microscope at Ex/Em = 594/610 nm

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

1. Prepare 1X iFluor™ 594-Phalloidin working solution:

Add 10 µL of iFluor™ 594-Phalloidin (ComponentA) to 10 mL of Labeling Buffer (Component B).

Note 1: The unused iFluor™ 594-Phalloidin stock solution (Component A) should be aliquoted and stored at -20 °C. Protect from light.

Note 2: Different cell types might be stained differently. The concentration of iFluor™ 594-Phalloidin working solution should be prepared accordingly.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2. Stain the cells:

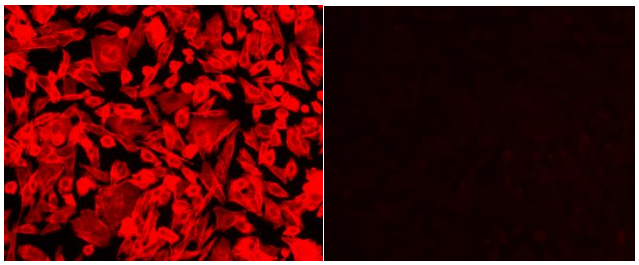
2.1 Perform formaldehyde fixation. Incubate the cells with 3.0–4.0 % formaldehyde in PBS at room temperature for 10–30 minutes.
Note: Avoid any methanol containing fixatives since methanol can disrupt actin during the fixation process. The preferred fixative is methanol-free formaldehyde.

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 Add 100 µL/well (96-well plate) of iFluor™ 594-Phalloidin working solution (from Step 1) into the fixed cells (from Step 2.2 or 2.3), and stain the cells at room temperature for 15 to 60 minutes.

2.5 Rinse cells gently with PBS 2 to 3 times to remove excess dye before plate sealing and imaging by using Texas Red channel (Ex/Em = 594/610 nm).



A **B**

Figure 1. Images of CPA cells fixed with formaldehyde and stained with Cell Navigator™ F-Actin Labeling Kit *Red Fluorescence* in a Costar black 96-well plate. A: Label the cells with 1X iFluor™ 594-Phalloidin for 30 min only. B: Treat the cells with phalloidin for 10 min, then stain them with 1X iFluor™ 594-Phalloidin for 30 min.

References

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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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