

## Cell Navigator™ F-Actin Labeling Kit

### \*Red Fluorescence\*

Ordering Information:	Storage Conditions:	Instrument Platform:
Product Number: 22663 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

### Introduction

Our Cell Navigator™ fluorescence imaging 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 red fluorescence. The kit uses a red fluorescent phalloidin conjugate that is selectively bound to F-actins. This red fluorescent phalloidin conjugate is a high-affinity probe for F-actins. When used at nanomolar concentrations, phalloidins 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 staining 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 TRITC filter (Ex/Em = 540/590 nm).

### Kit Components

Components	Amount
Component A: iFluor™ 546-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™ 546-Phalloidin solution → Stain the cells at RT for 15 to 60 min → Wash the cells → Examine the specimen under microscope at Ex/Em = 540/590 nm**

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

#### 1. Prepare 50X iFluor™ 546-Phalloidin stock solution:

Add 1 mL of methanol (Component C) into the iFluor™ 546-Phalloidin vial (Component A) to make 50X stock solution.

*Note: The unused iFluor™ 546-Phalloidin stock solution should be stored at -20 °C, and protected from light.*

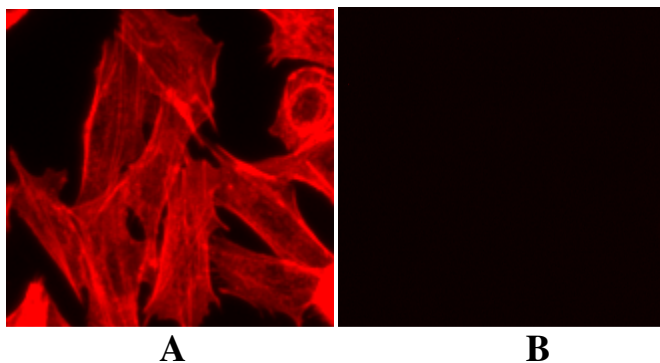
#### 2. Stain the cells:

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™ 546-Phalloidin working solution by diluting 200 µL of 50X iFluor™ 546-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™ 546-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 TRITC channel.



**Figure 1.** Images of CPA cells fixed with formaldehyde and stained with Cell Navigator™ F-Actin Labeling Kit \*Red Fluorescence\* in a 96-well Costar black plate A: Label the cells with 1X iFluor™ 546-Phalloidin for 30 min only. B: Treat the cells with phalloidin for 10 min, then stain them with 1X iFluor™ 546-Phalloidin for 30 min.

## References

1. Szczesna D, Lehrer SS (1993). The binding of fluorescent phalloidins to actin in myofibrils. *J Muscle Res Cell Motil*, 14(6), 594.
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.
5. Cooper JA. (1987). Effects of cytochalasin and phalloidin on actin. *J Cell Biol* 105, 1473.

**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**