F-Actin Staining Kit (Blue Fluorescence)

Catalog Number KA4115
500 assays
Version: 02

Intended for research use only
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Introduction

Background

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. Thus, 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 and maintenance of cell junctions and cell shape.

Principle of the Assay

F-Actin Staining Kit (Blue Fluorescence) is 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 of fixed cells in blue fluorescence. The kit uses a blue fluorescent phalloidin conjugate that is selectively bound to F-actins. The phalloidin conjugate has Ex/Em = 350/450 nm, compatible with DAPI filter set that comes with most of fluorescence microscopes. It is a high-affinity probe 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.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component A: iFluor 350-Phalloidin</td>
<td>1 vial</td>
</tr>
<tr>
<td>Component B: Labeling Buffer</td>
<td>50 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

✓ Keep in freezer
✓ Protect from light
✓ Avoid repeated freeze-thaw cycles

Precautions for Use

For research use only.
Assay Protocol

Reagent Preparation

✓ Prepare 1X iFluor 350-Phalloidin working solution:

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

   Note 1: The unused iFluor 350-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 350-Phalloidin working solution should be prepared accordingly.

Assay Procedure

✓ Stain the cells

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 (from Step 2) 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 (from Step Prepare 1X iFluor 350-Phalloidin working solution ) into the fixed cells (from Step 2 or 3), 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 and imaging by using DAPI channel.

✓ Summary

1. Prepare samples (microplate wells)
2. Remove the liquid from the plate
3. Add 100 µL/well of iFluor 350-Phalloidin solution
4. Stain the cells at RT for 15 to 60 minutes
5. Wash the cells
6. Examine the specimen under microscope at Ex/Em = 350/450 nm

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

Calculation of Results

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

☑ Typical Data

Figure 1. Images of CPA cells fixed with formaldehyde and stained with F-Actin Staining Kit (Blue Fluorescence) in black 96-well plate A: Label the cells with 1X iFluor 350-Phalloidin for 30 minutes only. B: Treat the cells with phalloidin for 10 minutes, then stain them with 1X iFluor 350-Phalloidin for 30 minutes.
References
