

Cell Explorer™ Fixed Cell Labeling Kit

Orange Fluorescence

| Ordering Information: | Storage Conditions: | Instrument Platform: |
|-----------------------------------|--|-------------------------|
| Product Number: 22602(500 Assays) | Keep in freezer Protect from moisture and light | Fluorescence microscope |

Introduction

Our Cell Explorer™ Fixed Cell Labeling kits are a set of tools for labeling cells for fluorescence microscopic investigations of cell functions. The effective labeling of cells provides a powerful method for studying cellular events in a spatial and temporal context. This particular kit is designed to uniformly label fixed mammalian cells in Orange fluorescence for long term microscopic examination. The kit uses a proprietary orange fluorescent dye that is more fluorescent upon binding to cellular components. The fluorescent dye used in the kit is quite photostable so that the images can be repeatedly examined. The kit provides all the essential components with an optimized cell-labeling protocol. 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 = 547/573 nm).

Kit Components

| Components | Amount |
|-------------------------------|-----------------|
| Component A: Stain It™ Orange | 1 vial |
| Component B: DMSO | 1 vial (200 µL) |

Assay Protocol

Brief Summary

Prepare Samples (microplate wells) → Remove the liquid from the plate → Add 100 µL/well of Stain It™ Orange solution → Stain the cells at RT for 10 min to hours → Wash the cells → Examine the specimen under microscope at Ex/Em = 547/573 nm

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

1. Prepare 500X Stain It™ Orange stock solution:

Add 100 µL DMSO (Component B) into the Stain It™ Orange vial (Component A) to make 500X stock solution.

Note: The unused Stain It™ Orange stock solution should be divided into single use aliquots and stored at -20°C. Avoid repeated freeze/thaw cycles.

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.
- 2.2 Rinse the fixed cells 2–3 times in PBS.
- 2.3 Prepare 1X Stain It™ Orange working solution by diluting 20 µL of 500X Stain It™ Orange stock solution (from Step 1) into 10 mL of PBS.
Note: A series concentrations (0.25X to 2.5X) of Stain It™ Orange should be used to get the the desired staining concentration for the cell line of interest.
- 2.4 Add 100 µL/well (96-well plate) of 1X Stain It™ Orange working solution (from Step 2.3) into the fixed cells (from Step 2.1), and stain the cells at room temperature for 10 minutes up to several hours.

2.5 Rinse cells gently with PBS several times to remove excess dye before plate sealing and imaging.

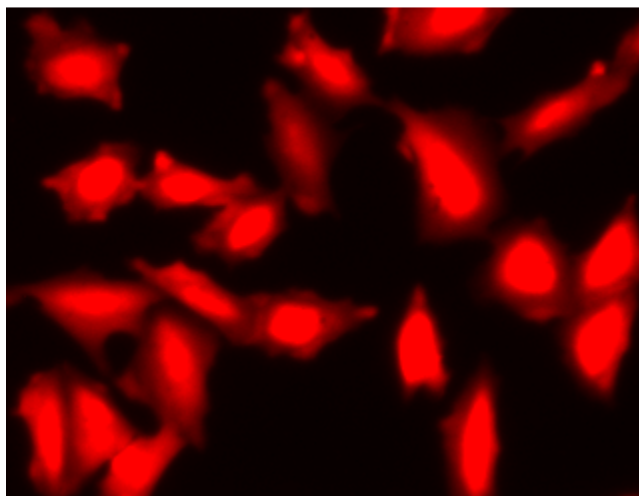


Figure 1: Image of CHO cells fixed with formaldehyde and stained with Cell Explorer™ Fixed Cell Labeling Kit *Orange Fluorescence* in a 96-well Costar black plate

References

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2. Lee S, Howell BJ. (2006) High-content screening: emerging hardware and software technologies. *Methods Enzymol*, 414, 468.
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4. Hudson CC, Oakley RH, Sjaastad MD, Loomis CR. (2006) High-content screening of known G protein-coupled receptors by arrestin translocation. *Methods Enzymol*, 414, 63.
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6. Giuliano KA. (2007) Optimizing the integration of immunoreagents and fluorescent probes for multiplexed high content screening assays. *Methods Mol Biol*, 356, 189.

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 if you have any questions.