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

Cell Explorer™ Fixable Dead Cell Labeling kits are a set of tools for labeling cells for fluorescence microscopic and flow cytometric investigations of cell functions. This particular kit is designed to uniformly label dead mammalian cells in orange fluorescence for microscopic examination and flow cytometry analysis.

The proprietary orange dye used in the kit becomes more fluorescent upon binding to cellular components. It is quite photostable, thus the images can be repeatedly examined. With the spectral properties almost identical to those of Cy3® or Alexa Fluor® 555 (Cy3® or Alexa Fluor® 555 are the trademarks of GE Healthcare and Invitrogen respectively), it can be conveniently used with the common fluorescence instruments equipped with the light sources and filters for Cy3® or Alexa Fluor® 555. This Cell Explorer™ Fixable Dead Cell Labeling Kit provides all the essential components with an optimized cell labeling protocol. It is an effective tool for labeling dead cells and preserving the fluorescent images. The kit can be used for both flow cytometry analysis and fluorescence microscopic investigations of cell functions by using a Cy3® or Alexa Fluor® 555 filter set (Ex/Em = 540/590 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 minutes to hours → Wash the cells → Examine the specimen under microscope at Ex/Em = 547/573 nm

Note: Thaw all the components at 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 Stain It™ Orange 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 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.

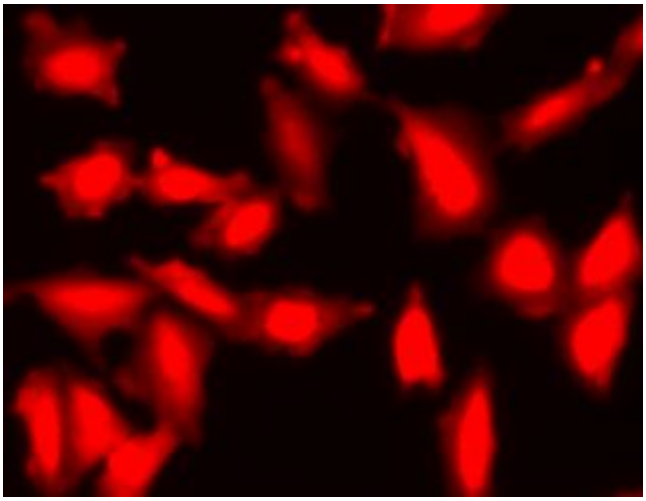

Figure 1: Image of CHO cells fixed with formaldehyde and stained with Cell Explorer™ Fixable Dead Cell Labeling Kit *Orange Fluorescence* in a Costar black 96-well plate

References

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