

**ab139489 –
CytoPainter
ER/Lysosome/Nuclear Staining
Reagent**

Instructions for Use

Designed to detect lysosomes, endoplasmic reticulum and nucleic acids by microscopy.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

ab139489 CytoPainter ER/Lysosome/Nuclear Staining Reagent is optimal for use in demanding cell analysis applications involving confocal microscopy, flow cytometry, microplate readers and HCS/HTS, where consistency and reproducibility are required.

2. Product Overview

ab139489 contains a mixture of cell-permeable red fluorescent endoplasmic reticulum dye, green fluorescent lysosomal dye and blue fluorescent nucleic acid dye. The staining pattern arising from the combination of these three dyes permits visualization of the target organelles by fluorescence/confocal microscopy. The reagent, supplied as a 500X solution, is sufficient for 1000 microscopy assays. The single-tube format makes this multi-organelle stain reagent easy to use.

3. Components and Storage

A. Kit Contents

Item	Quantity	Storage Temperature
Organelle Reagent IV	200 μ L	-80°C

Reagents provided in the kit are sufficient for approximately 1000 microscopy assays using either live, adherent cells or cells in suspension.

B. Storage and Handling

Upon receipt, the kit should be stored -80°C, protected from light. Avoid repeated freezing and thawing.

C. Additional Materials Required

- Standard fluorescence microscope
- Calibrated, adjustable precision pipets, preferably with disposable plastic tips
- Adjustable speed centrifuge with swinging buckets (for suspension cultures)
- Glass microscope slides
- Glass cover slips (18 x 18 mm)
- Deionized water
- Anhydrous DMSO (optional).
- Growth medium (e.g. Dulbecco's Modified Eagle medium, D-MEM)
- Paraformaldehyde (optional, for fixation)
- Triton X-100 (optional, for permeabilization)

4. Pre-Assay Preparation

NOTE: Allow all reagents to thaw at room temperature before starting with the procedures. Upon thawing, gently hand-mix or vortex the reagents prior to use to ensure a homogenous solution. Briefly centrifuge the vials at the time of first use, as well as for all subsequent uses, to gather the contents at the bottom of the tube.

A. Reagent Preparation

Mix 2 μ L of Organelle Reagent IV in 1 mL of buffer of choice. This volume is sufficient for 10 assays and may be scaled according to need.

5. Assay Protocol

Wide Field Fluorescence/Confocal Microscopy:

A. Staining Live, Adherent Cells

1. Grow cells directly onto glass slides or polystyrene tissue culture plates until ~80% confluent via standard tissue culture practices.
2. Remove growth media.
3. Dispense the freshly diluted staining solution in a volume sufficient for covering the cell monolayer.
4. Protect samples from light and incubate for 30 minutes at 37°C.

5. Remove the excess staining solution and, if necessary, add a few drops of buffer to prevent the cells from drying out.
6. Cover cells with a glass cover slip and observe under a fluorescence/confocal microscope with a filter set for DAPI (Ex/Em: 350/470nm), Texas Red (Ex/Em: 540/605 nm) and GFP/FITC (Ex/Em: 488/514 nm).

B. Staining Live Cells Grown in Suspension

1. Grow the cells via standard tissue culture practices.
2. Collect about 1×10^5 cells. Centrifuge at $500 \times g$ for 5 minutes. Remove supernatant.
3. Re-suspend cells in a volume of the freshly diluted staining solution sufficient for covering the cell pellet.
4. Protect the samples from light and incubate for 30 minutes at 37°C .
5. Centrifuge at $500 \times g$ for 5 minutes. Remove supernatant.
6. Re-suspend the cells in $100 \mu\text{L}$ buffer.
7. Plate $10\text{-}15 \mu\text{L}$ of cells on a glass slide.
8. Cover cells with a glass cover slip and observe under a fluorescence/confocal microscope with a filter set for DAPI (Ex/Em: 350/470nm), Texas Red (Ex/Em: 540/605 nm) and GFP/FITC (Ex/Em: 488/514 nm).

Wavelength Maxima:

Endoplasmic Reticulum(Red): Excitation: 580 nm Emission: 677 nm

Lysosomal (Green): Excitation: 481 nm Emission: 544 nm

Nuclear (Blue): Excitation: 350 nm Emission: 461 nm

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