

# **ab139482 – CytoPainter ER Staining Kit – Red Fluorescence**

## **Instructions for Use**

Designed to detect Human endoplasmic reticulum by microscopy.

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



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# 1. Introduction

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Fluorescence co-localization imaging with green fluorescent protein (GFP)-tagged proteins is a powerful approach for determining the targeting of molecules to intracellular compartments and for screening of their associations and interactions. However, to date, photoconversion of red fluorescent dyes to green fluorescent ones and metachromatic artifacts, wherein fluorescent dyes emit both in the red and green regions of the spectrum, have led to spurious results in GFP co-localization experiments. Additionally, many organelle targeting probes photobleach rapidly, are subject to quenching upon concentration in organelles, are highly toxic, or only transiently associate with the target organelle, requiring imaging within a minute or two of dye addition.

Consequently, Red Detection Reagent, a new red-emitting, cell-permeable small molecule organic probe that spontaneously localizes to live or fixed endoplasmic reticula, was developed. The CytoPainter ER Staining kit can be readily used in combination with other common UV and visible light excitable organic fluorescent dyes and various fluorescent proteins in multicolour imaging and detection applications. The dye emits in the Texas Red region of the visible light spectrum, and is highly resistant to photobleaching, concentration quenching and photoconversion.

## 2. Product Overview

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ab139482 contains a novel endoplasmic reticulum-selective dye suitable for live cell, or detergent-permeabilized aldehyde-fixed cell staining. Micromolar concentrations of Red dye are sufficient for staining mammalian cells, as validated with human cervical carcinoma cell line, human T-lymphocyte cell line, Jurkat, HeLa and human bone osteosarcoma epithelial cell line, U2OS.

ab139482 is specifically designed for use with GFP-expressing cell lines, as well as cells expressing blue, cyan or yellow fluorescent proteins (BFPs, CFPs, YFPs). Additionally, the kit is suitable for use with live or post-fixed cells in conjunction with probes, such as labeled antibodies, or other fluorescent conjugates displaying similar spectral properties as fluorescein, or coumarin. A nuclear counterstain is provided to highlight this organelle as well.

### 3. Assay Summary

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Thaw components, prepare reagents; warm to room temperature



Prepare cells



Add Dual Detection Reagent for 15-30 minutes at 37°C



Wash cells in Assay Buffer



Analyze stained cells by wide-field fluorescence or confocal microscopy

## 4. Components and Storage

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### A. Kit Contents

| Item                        | Quantity   | Storage Temperature |
|-----------------------------|------------|---------------------|
| Red Detection Reagent       | 50 $\mu$ L | -20°C               |
| Hoechst 33342 Nuclear Stain | 50 $\mu$ L | -20°C               |
| 10X Assay Buffer            | 15 mL      | -20°C               |

Reagents provided in the kit are sufficient for approximately 500 microscopy assays using either live, adherent cells or cells in suspension.

### B. Storage and Handling

Upon receipt, the kit should be stored upright at  $\leq -20^{\circ}\text{C}$ , or  $-80^{\circ}\text{C}$  for long term storage, 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).

## 5. Pre-Assay Preparation

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*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

#### 1. 1X Assay Buffer

Allow the 10X Assay Buffer to warm to room temperature. Make sure that the reagent is free of any crystallization before dilution. Prepare enough 1X Assay Buffer for the number of samples to be assayed by diluting each milliliter (mL) of the 10X Assay Buffer with 9 mL of deionized water.



## **2. 3.7% Formaldehyde Solution**

The following procedure is for preparation of 10 mL of 3.7% formaldehyde solution. Dilute 0.37 gram paraformaldehyde to a final volume of 10 mL with 1X Assay Buffer. Mix well.

## **3. 0.1% Triton X-100 (optional)**

The following procedure is for preparation of 10 mL of 1% Triton X-100 solution: Dilute 10  $\mu$ L Triton X-100 to a final volume of 10 mL with 1X Assay Buffer or 1X Assay Buffer containing 2% serum. Mix well.

## **4. Dual Detection Reagent**

The concentration of Red Detection Reagent for optimal staining will vary depending upon the application. Suggestions are provided to use as guidelines, through some modifications may be required depending upon the particular cell type employed and other factors such as the permeability of the dye to the cells or tissues. To reduce potential artifacts from overloading of the cells, the concentration of the dye should be kept as low as possible.

Prepare a sufficient amount of Detection Agent for the number of samples to be assayed as follows: For every milliliter of 1X Assay Buffer (see preparation in step 1) or 1X Assay Buffer containing 2% serum, add 1  $\mu$ L of Red Detection Reagent and 1  $\mu$ L of Hoechst 33342 Nuclear Stain.

**NOTE:**

- a) *The dyes may be combined into one staining solution or each may be used separately, if desired.*
- b) *The Hoechst 33342 Nuclear Stain can be diluted further if its staining intensity is much stronger than the red endoplasmic reticulum stain, Red Detection Reagent.*
- c) *When staining BFP- or CFP-expressing cells, the Hoechst 33342 Nuclear Stain should be omitted due to its spectral overlap with these fluorescent proteins.*

## **6. Assay Protocol**

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### **A. Staining Live, Adherent Cells**

1. Grow cells on 18 x 18 mm coverslips, or tissue culture treated slides, inside a Petri dish filled with the appropriate culture medium. When the cells have reached the desired level of confluence, carefully remove the medium.
2. Dispense a sufficient volume of Red Detection Reagent (Section 4A) to cover the monolayer of cells (around 100 $\mu$ L of labelling solution for cells grown on an 18 x18 mm coverslip).
3. Protect samples from light and incubate for 15-30 minutes at 37°C.

4. Wash the cells with 100  $\mu$ L 1X Assay Solution. Remove excess buffer and place coverslip on slide.
5. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard Rhodamine or Texas Red filter set for imaging the endoplasmic reticulum. Optionally, image the nucleus using a DAPI filter set and the GFP-tagged protein using a GFP/FITC filter set.

## **B. Staining Live Cells Grown in Suspension**

1. Centrifuge cells for 5 minutes at 400 x g at room temperature (RT) to obtain a cell pellet.
2. Carefully remove the supernatant by aspiration and dispense sufficient volume of Dual Detection Reagent (see Section 4A) to cover the dispersed cell pellet.
3. Protect samples from light and incubate for 15-30 minutes at 37°C.
4. (Optional) Wash the cells with 100  $\mu$ L 1X Assay Buffer. Remove excess buffer. Resuspend cells in 100  $\mu$ L 1X Assay Buffer, then apply the cells to a glass slide and overlay with a coverslip.
5. Analyze the stained cells by wide-field fluorescence or confocal microscopy (60X magnification recommended). Use a standard Rhodamine or Texas Red filter set for imaging the endoplasmic reticulum. Optionally, image the nucleus using a DAPI filter set and the GFP/FITC protein filter set.

### **C. Staining of Aldehyde-Fixed and Detergent-Permeabilized Live Cells with Red Detection Reagent**

The Red Detection Reagent is capable of staining fixed and permeabilized cells. Fixation and permeabilization makes it possible to probe for other intracellular structures by conventional immunofluorescence labeling methods.

1. Wash the cells in 1x Assay Buffer (from Section 4A).
2. Carefully remove the buffer covering the cells, and replace it with freshly prepared 3.7% formaldehyde solution.
3. Incubate the cells at 37°C for 10 minutes.
4. After fixation, rinse the cells several times in 1X Assay Buffer.
5. (Optional) If cells are to be labeled with an antibody, permeabilization step is recommended to enhance the antigen's accessibility. This is done by incubating the fixed cells in 0.1% Triton X-100 (Section 4A) at room temperature for 1 minute.
6. Following permeabilization, rinse the cells with 1X Assay Buffer.
7. Perform staining as recommended for adherent or suspension cells.

**NOTE:** *When performing standard immunofluorescence staining protocols using a Texas Red-, Rhodamine-, or Coumarin-labeled*

*antibodies, or equivalent, administer post-fixation according to manufacturer instructions.*

#### **D. Aldehyde Fixation and Detergent permeabilization of Stained Live Cells**

Live cells stained with Red Detection Reagent may be fixed with formaldehyde and permeabilized with Triton X-100. Fixation and permeabilization makes it possible to probe for other intracellular structures by conventional immunofluorescence labeling methods. The Red Detection Reagent dye is retained following fixation and permeabilization using the protocol described below.

1. Wash the Red-stained cells with 1x Assay Buffer (Section 4A).
2. Carefully remove the buffer covering the cells, and replace it with freshly prepared 3.7% formaldehyde solution.
3. Incubate the cells at 37°C for 10 minutes.
4. After fixation, rinse the cells several times in 1X Assay Buffer.
5. (Optional) If the cells are to be labeled with an antibody, permeabilization step is recommended to enhance the antigen's accessibility. This is done by incubating the fixed cells in 0.1% Triton X- 100 (from Section 4A) at room temperature for 1 minute.

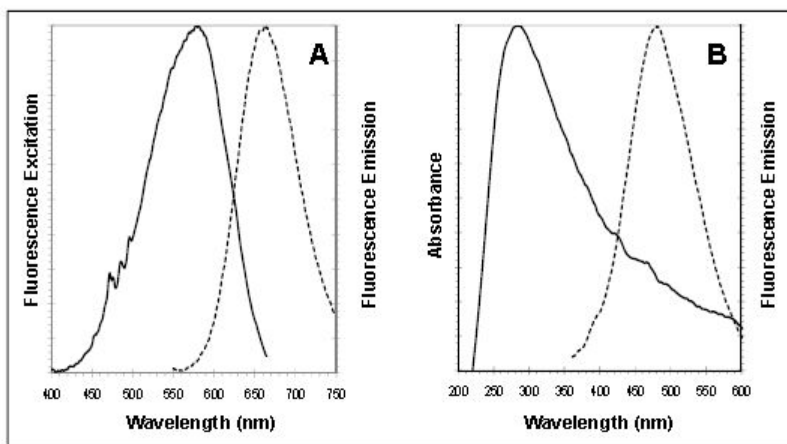
**NOTE:** *The staining will not be as intense after fixation of cells. It is recommended to fix prior to staining cells.*

## 7. Data Analysis

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### Filter set selection

The selection of optimal filter sets for a fluorescence microscopy application requires matching the optical filter specifications to the spectral characteristics of the dyes employed in the analysis. Consult the microscope or filter set manufacturer for assistance in selecting optimal filter sets for your microscope.



**Figure 1.** Fluorescence excitation and emission spectra for Red dye (panel A) and absorbance and fluorescent emission spectra for

Hoechst 33342 dye (panel B). All spectra were determined in 1X Assay Buffer.

Endoplasmic reticula are subcellular organelles found in eukaryotic cells, responsible for sorting most of the proteins and lipids of the cell. In addition to being a live cell-permeable dye, Red dye is also partially retained during or after cell fixation and detergent permeabilization.

Red dye has been shown to co-localize with EGFP-calreticulin chimeric protein in a transduced HeLa cell line. Typically, intense red fluorescent staining of the endoplasmic reticulum in the perinuclear region of mammalian cells is readily apparent using Red dye. The Red dye co-localizes with the EGFP-calreticulin signal, demonstrating selectivity for endoplasmic reticula.

## 8. Troubleshooting

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| <b>Problem</b>                                     | <b>Reason</b>  | <b>Solution</b>   |
|--|--|---|
| Endoplasmic reticula are not sufficiently stained. | Very low concentration of Red dye was used or dye was incubated with the cells for an insufficient length of time. | Either increase the labelling concentration or increase the time allowed for the dye to accumulate in the endoplasmic reticula. |
| Precipitate is seen in the 10X Assay Buffer        | Precipitate forms at low temperatures.   | Allow solution to warm to room temperature or 37°C, then vortex to dissolve all precipitate.                                    |



|   |   |   |
|---|---|---|
| <p>Blue nuclear counterstain is too bright compared to the red endoplasmic reticulum stain.</p> | <p>Different microscopes, cameras and filters may make some signals appear very bright.</p> | <p>Reduce the concentration of the nuclear counterstain or shorten the exposure time.</p>   |
| <p>Cells do not appear healthy</p>  | <p>Some cells require serum to remain healthy.</p>  | <p>Add serum to stain and wash solutions. Serum does not affect staining. Normal amounts of serum added range from 2% to 10%.</p> |





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