Product Manual

ER-ID® Green Assay Kit

Catalog #: ENZ-51025-K500

For detection of endoplasmic reticulum by microscopy

500 assays

NOTE: This version contains a change to shipping condition of product.

For the latest product information, including support documentation, visit us online:

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Product Manual

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NOTES:

Carefully note the handling and storage conditions of each kit component.
Please read entire booklet before proceeding with the assay.
Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Enzo Life Sciences' ER-ID® Green Assay Kit contains a novel endoplasmic reticulum-selective dye suitable for live cell, or detergent-permeabilized aldehyde-fixed cell staining. Micromolar concentrations of ER-ID® Green 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.

One important application of ER-ID® Green dye is in fluorescence co-localization imaging with red fluorescent protein (RFP)- or orange fluorescent protein (OFP)-tagged proteins, 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 fluorescent dyes to other colors and metachromatic artifacts, wherein fluorescent dyes emit both in the red (or orange) and green regions of the spectrum, have led to spurious results in co-localization experiments.[1,2] 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.[3,4] Consequently, ER-ID® Green dye, a new green-emitting, cell-permeable small molecule organic probe that spontaneously localizes to live or fixed endoplasmic reticula, was developed. ER-ID® Green dye can be readily used in combination with other common UV and visible light excitable organic fluorescent dyes and various fluorescent proteins in multi-color imaging and detection applications. The dye emits in the FITC region of the visible light spectrum, and is resistant to photo-bleaching, concentration quenching and photoconversion.

The ER-ID® Green Assay Kit is specifically designed for use with RFP- or OFP-expressing cell lines, as well as cells expressing blue or cyan fluorescent proteins (BFPs, CFPs). 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 Texas Red, or coumarin. A nuclear counterstain is provided to highlight this organelle as well.

SAFETY WARNINGS & PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.


Handle with care

- This product is for research use only and is not intended for diagnostic purposes.
- The ER-ID® Green Detection Reagent contains DMSO which is readily absorbed through the skin. It is harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Observe appropriate precautions when handling.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. All blood components and biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.


Protect from light

- To avoid photobleaching, perform all manipulations in low light environments or protected from light by other means.

REAGENTS SUPPLIED AND STORAGE


Avoid freeze / thaw cycles

All reagents are shipped on blue ice (-20°C). Upon receipt, the kit should be stored upright at ≤-20°C, protected from light. When stored properly, these reagents are stable for at least twelve months. **Avoid repeated freezing and thawing**.

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

Reagent	Quantity
ER-ID® Green Detection Reagent	50µl
Hoechst 33342 Nuclear Stain	50µl
10X Assay Buffer	15ml

Reagents require separate storage conditions.

OTHER MATERIALS NEEDED

1. Standard fluorescence microscope
2. Calibrated, adjustable precision pipetters, preferably with disposable plastic tips
3. Adjustable speed centrifuge with swinging buckets (for suspension cultures)
4. Glass microscope slides
5. Glass cover slips
6. Deionized water
7. Anhydrous DMSO (optional)
8. Growth medium (e.g., Dulbecco's Modified Eagle Medium, D-MEM)
9. Paraformaldehyde (optional, for fixation)
10. Triton X-100 (optional, for permeabilization)

METHODS AND PROCEDURES

***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.*

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 9ml of deionized water.

2. 3.7% FORMALDEHYDE SOLUTION

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

3. 0.1% TRITON X-100 (OPTIONAL)

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

4. DUAL DETECTION REAGENT

The concentration of ER-ID® Green dye for optimal staining will vary depending upon the application. Suggestions are provided to use as guidelines, though 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 sufficient amount of Dual Detection Reagent for the number of samples to be assayed as follows: For every milliliter of 1X Assay Buffer (see preparation in step 1, above) or 1X Assay Buffer containing 2% serum, add 1µl of ER-ID® Green Detection Reagent and 1µl 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 green endoplasmic reticulum stain, ER-ID® Green.
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.

STAINING LIVE, ADHERENT CELLS

1. Grow cells on cover slips, 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 sufficient volume of Dual Detection Reagent (see step 4 in section of REAGENT PREPARATION) to cover the monolayer cells (for example, ~100µl of labeling solution for cells grown on an 18 X 18 mm glass slide).
3. Protect samples from light and incubate for 15 to 30 minutes at 37°C.
4. Wash the cells with 100µl 1X Assay Buffer. 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 FITC filter set for imaging the endoplasmic reticulum. Optionally, image the nucleus using a DAPI filter set and the RFP-tagged protein using a Texas Red or Rhodamine filter set.

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 step 4 in section of REAGENT PREPARATION) to cover the dispersed cell pellet.
3. Protect samples from light and incubate for 15 to 30 minutes at 37°C.
4. (Optional) Wash the cells with 100µl 1X Assay Buffer. Remove excess buffer. Resuspend cells in 100µl 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 FITC filter set for imaging the endoplasmic reticulum. Optionally, image the nucleus using a DAPI filter set and the RFP-tagged protein using a Texas Red or Rhodamine filter set.

STAINING OF ALDEHYDE-FIXED AND DETERGENT-PERMEABILIZED LIVE CELLS WITH ER-ID® GREEN DYE

The ER-ID® Green dye 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 step 1, in section of REAGENT PREPARATION).
2. Carefully remove the buffer covering the cells, and replace it with freshly prepared 3.7% formaldehyde solution (from step 2, in section of REAGENT PREPARATION.
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 (from step 3, in section of REAGENT PREPARATION) 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 (see sections of STAINING LIVE, ADHERENT CELLS and STAINING LIVE CELLS GROWN IN SUSPENSION).

***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.*

ALDEHYDE FIXATION AND DETERGENT PERMEABILIZATION OF STAINED LIVE CELLS

Live cells stained with ER-ID® Green dye 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 ER-ID® Green dye is retained following fixation and permeabilization using the protocol described below.

1. Wash the ER-ID® Green-stained cells with 1X Assay Buffer (from step 1, in section of REAGENT PREPARATION).
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 step 3, in section of REAGENT PREPARATION) 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 (see previous section).*

APPENDICES

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.

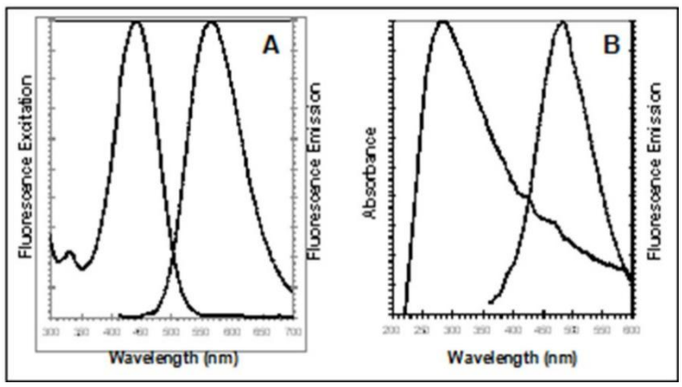

Figure 1. Absorbance and emission spectra for ER-ID® Green dye (panel A) and Hoechst 33342 dye (panel B). All spectra were determined in 1X Assay Buffer.

RESULTS

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, ER-ID® Green dye is also partially retained during or after cell fixation and detergent permeabilization.

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

REFERENCES

1. Freundt, Czapiga and Lenardo (2007) “Photoconversion of Lysotracker Red to a green fluorescent molecule” Cell Res. 17(11):956-958.
2. Nadrigny, Li, Kemnitz, Ropert, Koulakoff, Rudolph, Vitali, Giaume, Kirchhoff and Oheim (2007) “Systematic colocalization errors between acridine orange and EGFP in astrocyte vesicular organelles” Biophys J. 93(3):969-980.
3. Minamikawa, Sriratana, Williams, Bowser, Hill and Nagley (1999) Chloromethyl-X-rosamine (MitoTracker Red) photosensitises mitochondria and induces apoptosis in intact human cells. Journal of Cell Science 112, 2419-2430.
4. Scorrano, Petronilli, Colonna, Di Lisa and Bernardi (1999) Chloromethyltetramethylrosamine (Mitotracker Orange®) Induces the Mitochondrial Permeability Transition and Inhibits Respiratory Complex I: Implications for the mechanism of cytochrome c release” J. Biol Chem. 274, 35, 24657–24663.

TROUBLESHOOTING GUIDE

Problem	Potential Cause	Suggestion
Endoplasmic reticula are not sufficiently stained.	Very low concentration of ER-ID® Green dye was used or dye was incubated with the cells for an insufficient length of time.	Either increase the labeling 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.
Blue nuclear counterstain is too bright compared to the green endoplasmic reticulum stain.	Different microscopes, cameras and filters may make some signals appear very bright.	Reduce the concentration of the nuclear counterstain or shorten the exposure time.
Cells do not appear healthy	Some cells require serum to remain healthy.	Add serum to stain and wash solutions. Serum does not affect staining. Normal amounts of serum added range from 2% to 10%.

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