ER-Tracker™ Dyes for Live-Cell Endoplasmic Reticulum Labeling

ER-Tracker™ Blue-White DPX
ER-Tracker™ Green (glibenclamide BODIPY® FL)
ER-Tracker™ Red (glibenclamide BODIPY® TR)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Avoid freeze-thaw cycles
- Protect from light

Ex/Em:

- 374/430–640 nm (ER-Tracker™ Blue-White DPX dye)
- 504/511 nm (ER-Tracker™ Green dye)
- 587/615 nm (ER-Tracker™ Red dye)

Introduction

ER-Tracker™ dyes are cell-permeant, live-cell stains that are highly selective for the endoplasmic reticulum (ER). These dyes rarely stain mitochondria, unlike the conventional ER stain $DiOC_6(3)$, and staining at low concentrations does not appear to be toxic to cells. When cells are stained using the optimized protocol provided, staining patterns are partially retained after treatment with formaldehyde.

ER-Tracker™ Blue-White DPX (E12353) is a member of our Dapoxyl™ (DPX) dye family. DPX dyes have long emission wavelengths, high extinction coefficients, high quantum yields, and large Stokes shifts,[1] and are environment-sensitive probes—with increasing solvent polarity, the fluorescence maxima shift to longer wavelengths and the quantum yield decreases.

ER-Tracker™ Green and ER-Tracker™ Red dyes are the drug conjugates glibenclamide BODIPY® FL and glibenclamide BODIPY® TR, respectively. Glibenclamide (glyburide), a drug taken daily by millions of diabetic patients to correct hyperglycemia, has been used to investigate pancreatic β-cell activity and insulin secretion [2,3] and to study myocardial cell function and heart arrhythmia.[4,5] Glibenclamide binds to the sulphonylurea receptors of ATP-sensitive K^+ channels,[6] which are prominent on ER; the pharmacological activity of glibenclamide could potentially affect ER function. Variable expression of sulphonylurea receptors in some specialized cell types may result in non-ER labeling.

Materials

Contents

ER-Tracker™ Blue-White DPX dye (MW = 580.53) is packaged as a set of 20 separate vials, each containing 50 µL of a 1 mM stock solution in high-quality, anhydrous dimethylsulfoxide (DMSO). ER-Tracker™ Green dye (MW = 783.1) and ER-Tracker™ Red dye (MW = 915.23) are each provided as 100 µg of lyophilized material.

Storage and Handling

Upon receipt, these products should be stored with desiccant at ≤–20°C. AVOID REPEATED FREEZING AND THAWING; AVOID STORING THESE PRODUCTS IN A FROST-FREE FREEZER. When stored properly, these reagents are stable for at least six months.

Note: DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of these reagents in compliance with all pertaining local regulations.

Materials Required but not Provided

High-quality, anhydrous DMSO (for ER-Tracker™ Green and ER-Tracker™ Red dyes)

Spectral Characteristics

Spectral properties of the ER-Tracker™ dyes are listed in Table 1.

Table 1. Spectral characteristics of ER-Tracker™ dyes.

Dye	Ex/Em *	Filter
ER-Tracker™ Blue-White DPX dye	374/430–640 †	DAPI or UV longpass
ER-Tracker™ Green dye	504/511	FITC
ER-Tracker™ Red dye	587/615	TRITC

* Excitation (Ex) and emission (Em) maxima, in nm, measured in methanol.
† Dapoxyl™ dyes have highly environment-sensitive fluorescence, resulting in variable peak emission values.

Experimental Protocol

This protocol was optimized using bovine pulmonary artery endothelial cells and has been confirmed in other common cell lines. Recommendations for experimental protocols should be used as a starting point, and optimal labeling conditions for each cell type should be determined empirically.

Reagent Preparation

ER-Tracker™ Blue-White DPX is supplied as aliquots of a 1 mM stock solution in DMSO. Allow each vial to warm to room temperature before use, then briefly centrifuge to deposit the DMSO solution at the bottom of the vial.

ER-Tracker™ Green and ER-Tracker™ Red dyes are supplied as 100 µg of lyophilized material. Prepare a 1 mM stock solution of the appropriate dye: for ER-Tracker™ Green dye, dissolve the contents of the vial in 128 µL of DMSO; for ER-Tracker™ Red dye, dissolve the contents of the vial in 110 µL of DMSO. It is recommended that the 1 mM solution then be separated into aliquots and stored frozen with desiccant.

Cell Preparation and Staining

1.1 Prepare staining solution. Dilute the 1 mM stock solution to the final working concentration. We recommend working concentrations of 100 nM–1 µM for ER-Tracker™ Blue-White DPX and ~1 µM for ER-Tracker™ Green and ER-Tracker™ Red dyes. To minimize potential labeling artifacts, use the lowest dye concentrations possible. Best results are obtained when staining is performed in Hank's Balanced Salt Solution with calcium and magnesium (HBSS/Ca/Mg, Gibco cat. #14025-092) at 37˚C/5% CO_2.

1.2 Stain the cells. For adherent cells, remove the medium from the culture dish, rinse with HBSS, and add prewarmed staining solution. Incubate the cells for approximately 15–30 minutes at 37˚C. Replace the staining solution with fresh probe-free medium and view the cells using a fluorescence microscope. If the stained cells are to be fixed, refer to the fixation steps below for the appropriate dye.

Fixation and Permeabilization for ER-Tracker™ Blue-White DPX

2.1 Fix and permeabilize cells. ER-Tracker™ Blue-White DPX signal is only partially retained after formaldehyde fixation. Fix stained cells with 4% formaldehyde for 10–20 minutes at 37˚C. If additional staining will be performed, cells can be permeabilized with 0.2% Triton® X-100 for 10 minutes.

2.2 Wash and view cells. After cells are fixed, perform two 5-minute washes in any suitable buffer and view.

Fixation for ER-Tracker™ Green and ER-Tracker™ Red Dyes

3.1 Fix cells. If stained cells are to be fixed, fixation is recommended using 4% formaldehyde for 2 minutes at 37˚C.

3.2 Wash and view cells. After fixation, perform two 5-minute washes in any suitable buffer prior to mounting, viewing, or further staining. Permeabilization is not recommended; signal is not retained after permeabilization with Triton® X-100.

References

1. Photochem Photobiol 66, 424 (1997); **2.** Diabetes 52, 767 (2003); **3.** Biochem Pharmacol 67, 1437 (2004); **4.** Eur J Anaesthesiol 18, 811 (2001); **5.** Turk J Med Sci 30, 517 (2000); **6.** Br J Pharmacol 136, 995 (2002).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
E12353	ER-Tracker™ Blue-White DPX *for live-cell imaging* *1 mM solution in DMSO*	20 x 50 µL
E34250	ER-Tracker™ Red (BODIPY® TR glibenclamide) *for live-cell imaging*	100 µg
E34251	ER-Tracker™ Green (BODIPY® FL glibenclamide) *for live-cell imaging*	100 µg

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