

ER-Tracker™ Blue-White DPX (E-12353)

Quick Facts

Storage upon receipt:

- -20°C
- Avoid freeze-thaw cycles — Do not store in a frost-free freezer
- Protect from light

Introduction

The ER-Tracker™ Blue-White DPX (E-12353) is a member of our Dapoxyl™ dye family. The Dapoxyl (DPX) dyes have fluorescence properties that include long emission wavelengths, high extinction coefficients, high quantum yields and large Stokes shifts.¹ The DPX dyes are environment-sensitive probes — with increasing solvent polarity, the fluorescence maxima of these dyes shifts to longer wavelengths and the quantum yield decreases. These characteristics make the DPX dyes promising for biological applications.

The ER-Tracker Blue-White DPX dye is a photostable probe that is selective for the endoplasmic reticulum (ER) in live cells. ER-Tracker Blue-White DPX does not stain mitochondria (as does the conventional DiOC₆(3) “ER” stain), and staining at low concentrations does not appear to be toxic to cells. Moreover, our experiments indicate that staining patterns are retained after treatment with aldehyde fixatives such as formaldehyde, although at a reduced intensity. The high selectivity for ER makes ER-Tracker Blue-White DPX an excellent choice for use in combination with the mitochondrial stain, MitoTracker® Red CMXRos (M-7512).

Materials

Contents

The fluorescent ER-Tracker Blue-White DPX dye 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).

Storage and Handling

Upon receipt, these products should be stored desiccated at -20°C until required for use. **AVOID REPEATED FREEZING AND THAWING AND DO NOT STORE IN A FROST-FREE FREEZER.**

Before opening, the vial should be allowed to warm to room temperature and then briefly centrifuged in a microcentrifuge to deposit the DMSO solution at the bottom of the vial. Before

refreezing, seal the vial tightly. When stored properly, these stock solutions are stable for at least six months.

Properties

The absorption of the ER-Tracker Blue-White DPX is 374 nm in methanol. Since fluorescence of Dapoxyl dyes is highly environment-sensitive, the peak fluorescence emission is variable and ranges from 430 nm to 640 nm. Visualize fluorescence with a DAPI or UV longpass filter.

Cell and Tissue Loading

The protocol below is intended to serve as a guide for initial conditions. For best staining results, the procedure may require modification. In particular, the optimal concentration of probe may vary depending upon the characteristics of the cells used.

Cell Preparation and Staining

1.1 Dilute the 1 mM probe stock solution to the final working concentration in the growth medium or buffer of choice. For live-cell staining, we recommend working concentrations of 100 nM–1 µM for the ER-Tracker DPX probe. To reduce potential artifacts from overloading, the concentration of dye should be kept as low as possible. Incubation of cells in dye-free medium after staining reduces the fluorescent signal somewhat, but also helps to maintain cell viability.

1.2 For adherent cells, grow cells on coverslips inside a petri dish filled with the appropriate culture medium. When cells have reached the desired cell density, remove the medium from the dish and add the prewarmed (37°C) probe-containing medium (100 nM–1 µM). Incubate the cells for approximately 30 minutes under growth conditions appropriate for the particular cell type. Then replace the loading solution with fresh medium without stain and observe the cells using a fluorescence microscope fitted with an appropriate filter set (e.g., DAPI or UV longpass filter set). If the cells do not appear to be sufficiently stained, we recommend either increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the endoplasmic reticulum once the cells have been transferred to fresh medium. If the ER-Tracker DPX-stained cells are to be fixed and permeabilized, continue to steps 2.1–2.3.

1.3 For suspension cells, centrifuge to obtain a cell pellet and aspirate the supernatant. Resuspend the cells gently in prewarmed (37°C) probe-containing medium (100 nM–1 µM). Incubate the cells for 30 minutes under growth conditions appropriate for the particular cell type. Centrifuge the cells and resuspend in fresh, prewarmed medium. Again, if the cells are

not sufficiently stained, we recommend increasing the labeling concentration or increasing the time allowed for the dye to accumulate in the endoplasmic reticulum once the cells have been transferred to fresh medium. If the ER-Tracker DPX-stained cells are to be fixed and permeabilized, continue to steps 2.1–2.3.

Alternatively, suspension cells may be attached to coverslips that have been treated with Cell-Tak® (Collaborative Biomedical Products; Bedford, MA); in this case, see step 1.2.

Fixation and Permeabilization

2.1 A fraction of the ER-Tracker DPX probe appears to be retained after aldehyde fixation. Before fixation, cells must be

washed with phosphate-buffered saline (PBS) or any buffered saline solution. This step is especially important if the cells are attached to a Cell-Tak-coated coverslip or any other amine-containing surface treatment.

2.2 After staining with ER-Tracker Blue-White DPX dye, cells should be fixed with 3.7% formaldehyde for 10–20 minutes at 37°C. Cells can then be permeabilized with 0.2 % Triton® X-100 for 10 minutes.

2.3 After fixation and permeabilization, the cells should be rinsed in PBS and observed in PBS.

Reference

1. Photochem Photobiol 66, 424 (1997).

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

Cat #	Product Name	Unit Size
E-12353	ER-Tracker™ Blue-White DPX *1 mM solution in DMSO* *special packaging	20x50 µL

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — www.probes.com — for the most up-to-date information

Molecular Probes, Inc.

PO Box 22010, Eugene, OR 97402-0469
Phone: (541) 465-8300 • Fax: (541) 344-6504

Customer Service: 7:00 am to 5:00 pm (Pacific Time)
Phone: (541) 465-8338 • Fax: (541) 344-6504 • order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 465-8353 • Fax: (541) 465-4593 • tech@probes.com

Molecular Probes Europe BV

PoortGebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)
Phone: +31-71-5233431 • Fax: +31-71-5233419
eurotech@probes.nl

Molecular Probes' products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Several of Molecular Probes' products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 1999, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.