GFP Counterstain (BODIPY® TR Methyl Ester)

Catalog no. C34556

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
GFP counterstain (BODIPY® TR methyl ester)	1 mL	5 mM solution in DMSO	• Room temperature • Protect from light	When stored as directed this product is stable for 1 year.
Number of assays: Sufficient material is supplied to stain ~2,000 zebrafish embryos based on the protocol below.				
Approximate fluorescence excitation/emission maxima: GFP counterstain BODIPY® TR methyl ester **:** 598/625 in nm.				

Introduction

Many research and biotechnological applications require detailed three- and four-dimensional visualization of embryonic cells labeled with green-fluorescent protein (GFP) within their native tissue environments. Fluorescent counterstains that label all the cells in a living embryo provide a histological context for the GFP-expressing cells in the specimen.

GFP Counterstain (BODIPY® TR methyl ester) is an excellent counterstain for GFP-expressing cells and tissues. This dye readily permeates cell membranes and localizes in endomembranous organelles such as ER, the Golgi apparatus, and mitochondria, but does not appear to localize in the plasma membrane. These localization properties make the dye an ideal vital stain that can be used to reveal the:

- Location and shapes of cell nuclei
- Shapes of cells within embryonic tissues
- Boundaries of organ-forming tissues within the whole embryo

Furthermore, GFP Counterstain (BODIPY® TR methyl ester) staining is retained after paraformaldehyde fixation, and the dye does not appear to produce any teratogenic effects on embryonic development.[1] The emission spectra of enhanced GFP (EGFP) and BODIPY® TR methyl ester are well separated, with peaks at 508 nm and 625 nm, respectively (Figure 1), allowing simultaneous dual-channel confocal imaging without significant overspill of GFP fluorescence into the BODIPY® TR methyl ester detection channel.

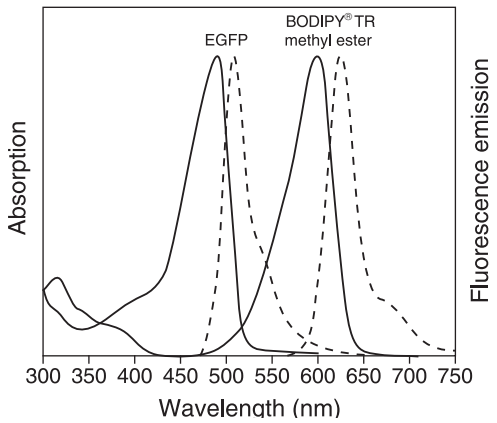

Figure 1. Normalized absorption (--) and fluorescence emission (----) spectra of EGFP and BODIPY® TR methyl ester.

Before Starting

Materials Required but Not Provided

- Appropriate buffer (for staining cultured cells)
- 4% formaldehyde (for staining cultured cells)

Caution

No data are available addressing the mutagenicity or toxicity of this reagent. Handle the DMSO stock solution with caution, as DMSO is known to facilitate the entry of organic molecules into tissues. Dispose of the stain in compliance with all pertaining local regulations.

Experimental Protocols

Staining Zebrafish Embryos

1.1 Add 5 µL of the GFP Counterstain (BODIPY® TR methyl ester) stock solution directly to 250 µL of embryo rearing medium.

The resulting staining solution contains 100 µM dye and 2% DMSO and is sufficient to simultaneously stain approximately 10 zebrafish embryos.

1.2 Stain embryos for 1 hour, then wash three times with plain embryo rearing medium or saline solution.

Staining Cultured Cells

1.1 Prepare a staining solution from the GFP Counterstain (BODIPY® TR methyl ester) stock solution.

For example, prepare a 1 µM staining solution by diluting 0.2 µL of the stock solution into 1 mL of buffer.

Note: We have used dye concentrations from 0.01 to 1.0 µM in Hanks' balanced salt solution containing 10 mM HEPES, pH 7.4, with the best results at ~0.1 µM. We have tested this protocol on MRC-5 human fibroblasts and bovine pulmonary aorta endothelial (BPAE) cells with generally similar results. Optimize the dye concentration for your particular cell type.

1.2 Incubate live cells in the staining solution for 10 minutes at 37°C.

1.3 Wash and resuspend the cells in balanced salt solution or medium.

1.4 Fix cells in 4% formaldehyde (see **Note** below).

Note: Permeabilization of cells or tissue with detergents, methanol, or acetone may remove the dye from the sample, altering the pattern or intensity of labeling.

Fluorescence Imaging

Filter sets suitable for Texas Red® dye are recommended for imaging samples labeled with BODIPY® TR methyl ester. For confocal laser-scanning microscopy (CLSM), excitation at 568 nm (argon/krypton-ion laser) or 561 nm (yellow diode laser) is recommended.

Reference

1. Personal communication, Mark Cooper,, University of Washington.

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

Cat. no.	Product Name	Unit Size
C34556	GFP Counterstain (BODIPY® TR Methyl Ester) *solution in DMSO*	1 mL

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