FluoroMyelin™ Fluorescent Myelin Stains

FluoroMyelin™ Green Fluorescent Myelin Stain (F34651)
FluoroMyelin™ Red Fluorescent Myelin Stain (F34652)

Quick Facts

Storage upon receipt:

- 4ºC
- Protect from light

Ex/Em:

- FluoroMyelin Green fluorescent myelin stain: 479/598 nm
- FluoroMyelin Red fluorescent myelin stain: 558/654 nm

Introduction

The FluoroMyelin™ Red and FluoroMyelin Green fluorescent myelin stains enable quick and selective labeling of myelin in brain cryosections. Visualization of myelin is useful for understanding myelin distribution, identifying brain structures and relative location of other labels, and mapping a particular section within the brain. Traditional methods require the use of antibodies, such as anti–myelin basic protein, or chromogenic (transmitted-light) methods, such as the Loyez method or Schmued's gold chloride technique, all of which are time consuming, requiring multiple steps over one to three days. The FluoroMyelin stains, in contrast, require only a single 20-minute labeling step plus washes. These stains can be used in combination with antibodies and other dyes, and with standard histochemical methods for cryosectioned material. They are believed to work via lipophilic affiliation, taking advantage of the high lipid content of myelin in axon sheaths, but they are more selective than traditional lipophilic dyes such as DiI. The lipophilic nature of FluoroMyelin stains does, however, lead to faint labeling of all cell membranes. In addition to being used on their own, FluoroMyelin stains can be combined with other dyes in a single labeling step as in our BrainStain™ Imaging Kit, which includes NeuroTrace® 530/615 red fluorescent Nissl stain and DAPI nucleic acid stain for three-color visualization.

Materials

Contents

The FluoroMyelin Green and FluoroMyelin Red stains are provided in a volume of 1 ml. Each is a 300X solution in water.

Storage

Upon receipt, the stains should be stored at 4°C, upright and protected from light. The stock solutions are stable for at least 6 months if stored properly.

Spectral Characteristics

A standard FITC filter set is suitable for imaging FluoroMyelin Green stain (excitation/emission maxima ~479/598 nm). There should be little or no bleedthrough into standard DAPI, TRITC, or far-red filter sets.

A standard TRITC or Texas Red® filter set is suitable for imaging FluoroMyelin Red stain (excitation ~558/654 nm). There should be little or no bleedthrough into standard DAPI, FITC, or far-red filter sets.

Protocol for Staining Mouse Brain Cryosections

This protocol has been optimized for staining 12 µm mouse brain cryosections on Superfrost Plus slides (Erie Scientific Co.), using 200 µl of staining solution within a PAP pen well at room temperature. Optimal staining conditions may vary slightly depending on sample conditions and should be determined by the user.

1. Rehydrate and permeabilize specimens. Bring the tissue sections on slides to room temperature, then rehydrate them in either PBS[1] or PBT (PBS + 0.2% TRITON X-100) for at least 20 minutes. Permeabilization with TRITON X-100 is not necessary for staining with FluoroMyelin stains, but is likely to be necessary for other counterstains or antibodies to be used.

2. Prepare staining solution. Prepare the staining solution by diluting the stock solution 300-fold into PBS.

3. Stain specimens. Flood the sections with staining solution and stain them for 20 minutes at room temperature.

4. Wash. When staining is complete, remove the solution, rinse in PBS, and wash 3 times for 10 minutes each with PBS.

5. Counterstain and mount. At this point the sections can be counterstained as necessary, or mounted with an aqueous antifade mounting medium such as ProLong® or ProLong Gold antifade reagent.

Note

1. PBS (phosphate-buffered saline) = 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate (Na_2HPO_4/KH_2PO_4), pH 7.4

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

Cat #	Product Name	Unit Size
F34651	FluoroMyelin™ Green fluorescent myelin stain *solution in water*	1 ml
F34652	FluoroMyelin™ Red fluorescent myelin stain *solution in water*	1 ml

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 Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — **probes.invitrogen.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

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

Technical Service: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

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