

## **BrainStain™ Imaging Kit (B34650)**

### **Quick Facts**

#### **Storage upon receipt:**

- $\leq -20^{\circ}\text{C}$
- Desiccation recommended
- Protect from light

#### **Ex/Em:**

- FluoroMyelin™ Green fluorescent myelin stain:  
479/598 nm
- NeuroTrace® 530/615 red fluorescent Nissl  
stain: 530/615 nm
- DAPI: 358/461 nm (when bound to DNA)

### **Introduction**

The BrainStain™ Imaging Kit enables three-color combinatorial labeling of myelin, neurons, and nuclei in brain cryosections in a single 20-minute staining step. The kit contains novel stains that can be used together in one staining solution or separately, and replaces traditional methods that can take one to three days. Standard histochemical methods, such as immunohistochemistry, are compatible with these stains.

FluoroMyelin™ Green stain is a fluorescent, selective myelin stain that replaces traditional chromogenic (transmitted-light) methods, such as the Loyez method or Schmued's gold chloride technique, or the use of antibodies, such as anti-myelin basic protein (MBP), though anti-MBP is better for imaging finely resolved processes. FluoroMyelin Green stain is believed to work via lipophilic affiliation, taking advantage of the high lipid content of myelin in axon sheaths, but it is more selective than traditional lipophilic dyes such as DiI. The lipophilic nature of FluoroMyelin Green stain does, however, lead to faint staining of all cell membranes.

NeuroTrace® 530/615 red fluorescent Nissl stain selectively stains neuron cell bodies by binding to the rough endoplasmic reticulum of neuronal perikarya and dendrites (the "Nissl substance"). It replaces traditional fluorescent and chromogenic dyes such as cresyl violet, methylene blue, safranin-O, and toluidine blue-O, and has improved sensitivity.

DAPI is a widely used blue-fluorescent nuclear counterstain, which binds specifically to DNA.

### **Materials**

#### **Kit Contents**

- **FluoroMyelin Green fluorescent myelin stain**, 1 ml, 300X solution in water
- **NeuroTrace 530/615 red fluorescent Nissl stain**, 1 ml, 300X solution in DMSO
- **DAPI dihydrochloride**, 1 ml, 300X solution in water

#### **Storage and Handling**

Upon receipt, all dyes should be stored at  $\leq -20^{\circ}\text{C}$ , upright and protected from light. The stock solutions are stable for at least 6 months if stored properly. Caution: The DAPI and NeuroTrace stain should be handled with care, as they bind to nucleic acids and are known or potential mutagens. They should be disposed of safely and in accordance with applicable regulations. They can be removed from aqueous solutions by filtration through activated charcoal. The charcoal and adsorbed dye must then be disposed of in a safe and appropriate manner. The NeuroTrace stain should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues.

#### **Spectral Characteristics**

A standard FITC filter set is suitable for imaging FluoroMyelin Green stain (excitation/emission maxima  $\sim 479/598$  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 NeuroTrace 530/615 red fluorescent Nissl stain (excitation/emission maxima  $\sim 530/615$  nm). There should be little or no bleedthrough into standard DAPI, FITC, or far-red filter sets.

DAPI shows blue fluorescence upon binding DNA (excitation/emission maxima  $\sim 358/461$  nm) and can be imaged using a standard DAPI filter set.

### **Protocol for Staining Mouse Brain Cryosections**

This protocol has been optimized for staining 12  $\mu\text{m}$  mouse brain cryosections on Superfrost Plus slides (Erie Scientific Co.), using 200  $\mu\text{l}$  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. This protocol combines all three stains into one staining

solution and staining step, but the dyes can be applied in separate steps, with the same individual concentrations and staining times with buffer washes in between.

**1. Rehydrate and permeabilize specimens.** Bring the tissue sections on slides to room temperature, then rehydrate and permeabilize them in PBT (PBS<sup>1</sup> + 0.2% TRITON X-100) for at least 20 minutes.

**2. Prepare staining solution.** Prepare the three-color staining solution by diluting each stock solution 300-fold into PBS in a single vial.

**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 with PBT, and wash 3 times for 10 minutes each with PBT. Longer washing in PBT may increase the specificity of the NeuroTrace stain.

**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<sup>®</sup> or ProLong Gold antifade reagent.

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### Note

1. PBS (phosphate-buffered saline) = 137 mM NaCl, 2.7 mM KCl, 10 mM phosphate (Na<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>), pH 7.4

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

Cat #	Product Name	Unit Size
B34650	BrainStain™ Imaging Kit .....	1 kit

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### 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.

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#### 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)

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Fax: (541) 335-0238 • [probestech@invitrogen.com](mailto: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: [euinfo@invitrogen.com](mailto:euinfo@invitrogen.com)  
Technical Services: [euotech@invitrogen.com](mailto:euotech@invitrogen.com)

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