NeuroTrace™ Fluorescent Nissl Stains

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Ex/Em: See Table 1

Introduction

The Nissl substance, described by Franz Nissl more than one hundred years ago, is abundant in neuronal cells. Composed of ribosomal RNA associated with the rough endoplasmic reticulum in neuronal perikarya and dendrites, the Nissl substance reflects the unusually high protein synthesis capacity of neuronal cells. Various fluorescent or chromophoric "Nissl stains" have been used to stain the Nissl substance in tissue preparations, thereby identifying neuronal cells. Stains traditionally used for this purpose include cresyl violet, methylene blue, safranin-O and toluidine blue-O. In injured or regenerating neurons, the Nissl substance breaks apart and redistributes around the periphery of the cell body, one of several histological changes collectively known as chromatolysis. Therefore, Nissl stains are also useful markers for the physiological state of the neuron, particularly in dorsal root ganglion cells and primary motor neurons.

We have developed fluorescent Nissl stains that provide a wide spectrum of fluorescent colors for staining neurons (Table 1), either alone or in combination with immunofluorescence staining of specific proteins, and are more sensitive than the conventional dyes.

Materials

Contents

The NeuroTrace fluorescent Nissl stains are supplied in 1 mL unit sizes as solutions in DMSO.

Storage

Upon receipt, the stains should be stored at -20°C, desiccated and protected from light.

Handling

Before opening, each vial should be allowed to warm to room temperature and then briefly centrifuged in a microfuge to deposit the DMSO solution at the bottom of the vial. If particles of dye are present, they should be redissolved by briefly sonicating the tube or vortexing it vigorously after warming. **Caution:** No data are available addressing the mutagenicity or toxicity of NeuroTrace fluorescent Nissl stains. Because these reagents bind to nucleic acids, they should be treated as a potential mutagens and handled with appropriate care. The DMSO stock solutions should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Table 1. *Spectral characteristics of NeuroTrace fluorescent Nissl stains.*

Cat #	Fluorescent color	Ex *	Em *	Signal using various filter sets **				
				405 ± 20 445 ≥450	485 ± 11 505 535 ± 17.5	510 ± 11.5 540 ≥550	560 ± 20 590 610 ± 10	640 ± 10 660 682 ± 11
N-21479	blue	435	455	+++	+	+/-	-	-
N-21480	green	500	525	-	+++	+++	-	-
N-21481	yellow	515	535	-	+++	+++	+++	-
N-21482	red	530	615	-	-	++	+++	-
N-21483	deep red	640	660	-	-	- †	- †	++

* Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm. ** Spectral characteristics in nm of the excitation filter, dichroic mirror and emission filter are shown top to bottom for each filter set. Relative signal strength for each filter combination is indicated with pluses and minuses.
† High background fluorescence.

Disposal

NeuroTrace fluorescent Nissl stains should be disposed of safely and in accordance with applicable regulations. NeuroTrace fluorescent Nissl stains 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.

Protocol for Staining Mouse Brain Cryosections

The following protocol has been used successfully in our laboratories to stain mouse brain cryosections with the NeuroTrace fluorescent Nissl stains. We recommend using this protocol as a guide for staining other specimen preparations. If used in combination with antibody staining, we recommend performing the antibody staining first, followed by staining with the fluorescent Nissl stain. **Note:** Blocking solutions containing bovine serum albumin (BSA), nonfat dried milk or horse serum may quench the fluorescence of Nissl stains. A possible alternative to these reagents is 0.5% fish skin gelatin, which does not appear to quench the signal.

1. Prepare the cryosections using standard protocols and place on a slide.

2. Rehydrate the sections for at least 40 minutes in 0.1 M phosphate-buffered saline (PBS), pH 7.2.

3. Wash the sections for 10 minutes in PBS plus 0.1% Triton® X-100. This step permeabilizes the tissue and is required for optimal staining.

4. Wash the sections two times for 5 minutes each in PBS.

5. Dilute the NeuroTrace stain in PBS. Dilution factors from 20- to-300-fold have been used successfully. An optimal dilution factor should be determined empirically.

6. Apply approximately 200 µL of the diluted stain to the slide, so that the section is covered, and incubate for 20 minutes.

7. Remove the stain and wash the sections for 10 min in PBS plus 0.1% Triton X–100.

8. Wash the sections two times for 5 minutes each in PBS.

9. Wash the sections for 2 hours at room temperature or overnight at 4°C in PBS.

10. Counterstain as desired and wash.

11. Apply a suitable mounting medium and cover the sections with a coverslip. We recommend using the ProLong® Antifade Kit (P-7481) for mounting.

Note: When using the ProLong Antifade Kit, it is generally best to wait until the mounting medium has set (overnight at room temperature) before viewing the slides. The staining pattern should be well preserved for several weeks or longer if the slide is stored in the dark at 4°C or -20°C. However, over time, background staining may increase due to leaching of the dye into the mounting medium.

References

1. Neurosci Lett 184, 169 (1995).

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

Cat #	Product Name	Unit Size
N-21479	NeuroTrace™ 435/455 blue fluorescent Nissl stain *solution in DMSO*	1 mL
N-21483	NeuroTrace™ 640/660 deep-red fluorescent Nissl stain *solution in DMSO*	1 mL
N-21480	NeuroTrace™ 500/525 green fluorescent Nissl stain *solution in DMSO*	1 mL
N-21482	NeuroTrace™ 530/615 red fluorescent Nissl stain *solution in DMSO*	1 mL
N-21481	NeuroTrace™ 515/535 yellow fluorescent Nissl stain *solution in DMSO*	1 mL

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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.

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