Staining Brain Sections with Fluorescent Nissl Stains

Nissl staining is a commonly used histological technique to visualize neural tissue morphology. The method is based on the interaction of basic dyes with the nucleic acid content of cells. Due to intensive protein synthesis, the perikarya of neurons has abundant ribosomal RNA in the rough endoplasmic reticulum ('Nissl substance'), and cytoplasmic staining of neurons is much stronger than in nuclei. On this basis, stained neurons can be distinguished from glial cells, and therefore, Nissl staining is considered specific for detecting neurons.

Fluorescent Nissl Stains are cell-impermeant dyes that are nonfluorescent in the absence of nucleic acids but exhibit a significant fluorescence enhancement upon binding to RNA and DNA. We offer highly concentrated (1,000×) Fluorescent Nissl Stains with different spectral properties.

Handling and Disposal

Before opening, each vial should be warmed to room temperature and briefly centrifuged in a microfuge to drop the solution at the bottom of the vial. If any dye particles are present, redissolve them by briefly sonicating or vigorously vortexing the tube after warming.

Please note that there is currently no data on the mutagenicity or toxicity of Fluorescent Nissl Stains. Since these reagents bind to nucleic acids, they should be treated as potential mutagens and handled carefully. The DMSO stock solution requires special attention, as DMSO can enhance the absorption of organic molecules into tissues. As with all nucleic acid reagents, Fluorescent Nissl Stain solutions should be disposed of in accordance with local regulations.

Before you start

- If the Nissl staining is combined with immunochemistry, the antibody incubations are performed first, followed by staining with the Fluorescent Nissl Stain.
- Blocking solutions containing horse serum, bovine serum albumin, or nonfat dried milk may quench the signal of the Fluorescent Nissl Stain. 0.5% fish skin gelatin is a possible alternative to these reagents.

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Staining Brain Cryosections

1. Prepare the cryosections and collect them on a poly-L-lysine-covered slide. Let the sections dry.

   Important! Since when staining on glass, the dye diffuses only from one side of the specimen; we do not recommend using sections thicker than 25 µm for this purpose.

2. Fix sections with ice-cold buffered 4% paraformaldehyde for 15 minutes.
3. Wash the sections twice for 10 minutes each in 0.1 M phosphate-buffered saline (PBS), pH 7.4.
4. Permeabilize the sections in 0.1% TBS (PBS with 0.1% Triton® X-100) for 10 minutes at room temperature. This step is required for optimal staining.
5. Wash the sections two times for 5 minutes each in PBS.
6. To prepare the staining solution, add the dye concentrate to PBS at a ratio of 1:1,000 and mix thoroughly. An optimal dilution should be determined empirically.
7. Apply approximately 200 µL of the staining solution to the slide so that the section is fully covered, and incubate for 20 minutes at room temperature.
8. Remove the stain and wash the sections in 0.1% TBS for 10 minutes at room temperature.
9. Wash the sections twice for 5 minutes each in PBS.
10. For the best results, wash the sections in PBS additionally for 2 hours at room temperature or overnight at 4 °C.
11. Counterstain the sections if necessary and wash them in PBS.

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Staining Free-Floating Brain Sections

1. Prepare the fixed brain sections and collect them in the wells of the cell culture plate.
2. Wash the sections twice for 10 minutes each in PBS, pH 7.4.
3. Permeabilize the sections in 0.1% TBS for 10 minutes at room temperature. This step is required for optimal staining.
4. To prepare the staining solution, add the dye concentrate to PBS at a ratio of 1:1,000 and mix thoroughly. An optimal dilution should be determined empirically.
5. Add approximately 500 µL of the staining solution to each well and incubate for 20 minutes at room temperature.
6. Remove the stain and wash the sections in 0.1% TBS for 10 minutes at room temperature.
7. Wash the sections twice for 5 minutes each in PBS.
8. For the best results, wash the sections in PBS additionally for 2 hours at room temperature or overnight at 4 °C.
9. Counterstain the sections if necessary and wash them in PBS.
10. Collect the sections on poly-L-lysine-covered or other adhesive slides.

Mounting Coverslips

1. Apply a suitable mounting medium and cover the sections with a coverslip. We recommend to use our Lumimount Mounting Medium and detailed protocol for mounting coverslips.
2. The staining is well preserved for several weeks or longer if the slide is kept in the dark at 4 °C or -20 °C. However, the background may increase over time as the dye leaches into the mounting medium.

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