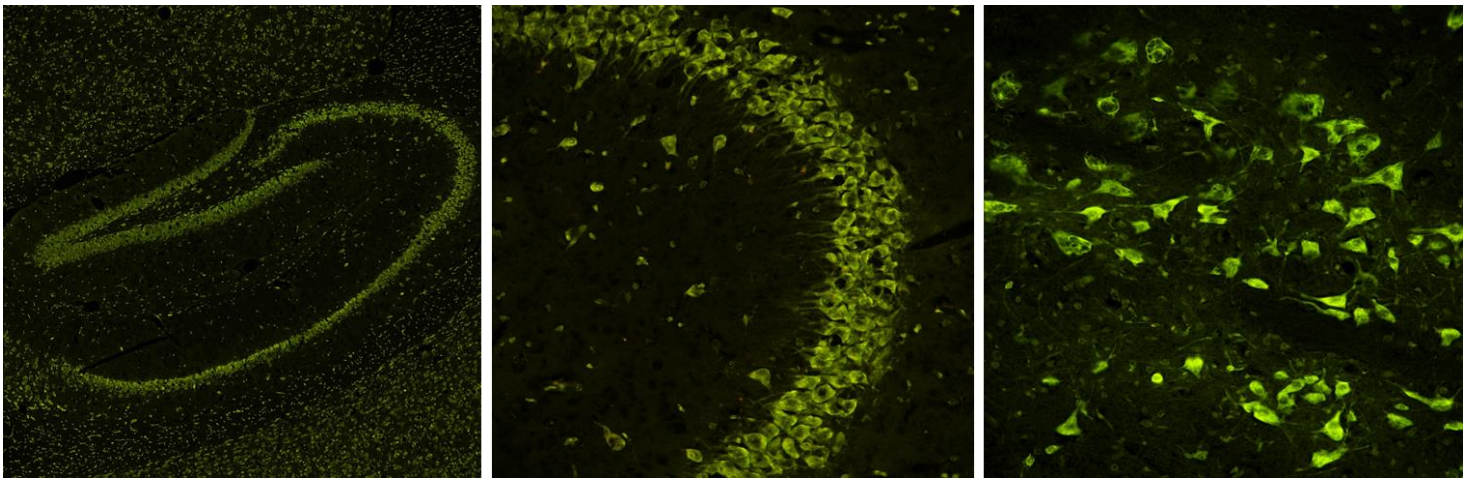
NisslFluor™ Green RTD™

A fluorescent Nissl stain for neurons

Catalog Number: TR-220-NGN



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Scientific Background

Nissl substance, also known as chromophilic substance, is a granular organelle found in the cytoplasm of neurons. It is named after Franz Nissl, a neurologist who discovered it in the late 19th century. The Nissl substance comprises rough endoplasmic reticulum (rER), RNA, and proteins. Ribosomes are the cell's protein factories, and they synthesize proteins used by the neuron for various functions, including cell growth, maintenance, and repair.

Nissl substance is most abundant in neurons' soma or cell body. It is also found in the proximal dendrites, but it is not present in the axon. The visibility, size, form, and distribution of Nissl substance can vary depending on the type of neuron and its activity level. For example, Nissl substances are typically more abundant in neurons actively synthesizing proteins, such as pyramidal cells and motor neurons. In pyramidal cells and motor neurons, Nissl bodies appear as large, basophilic chunks of material. These neurons have long axons and distant terminals that require maintenance. In contrast, Nissl bodies are dispersed in a powdered form in sensory ganglion cells with long axons.

Under normal conditions, Nissl staining bodies are evenly distributed in the soma and proximal dendritic regions. However, some healthy neurons may be concentrated near the plasmalemma. This outward dispersion is often a sign of neuronal injury, anoxia, or disease and is called central chromatolysis and can indicate damage to the neuron. For example, if the axon of a neuron is severed, the Nissl substance will disappear from the cell body (a process known as retrograde chromatolysis).

Thus, the Nissl substance is a valuable neuronal activity and damage marker. It can change in size and distribution in response to different stimuli and is often reduced in size and number in neurodegenerative diseases. For example, in Alzheimer's disease, the reduced size and amounts seen in patients are thought to contribute to the cognitive decline characteristic of the disease.

NisslFluor™ Green RTD™ Description

NisslFluor™ Green RTD™ is a ready-to-dilute fluorescent Nissl stain provided as a 10X, 40 mL liquid stock solution. The material is easily diluted with distilled water to a 1X working solution.

NisslFluor™ Green Nissl stain will stain the soma and proximal reaches of dendrites, cytoplasm, and nuclei of neurons a fluorescent green color under blue light excitation. Nissl substance is not normally found in the axon hillock or the axon itself.

NisslFluor™ Green will be of high contrast, resolution, and brightness and resistant to fading. Visible under blue light (450-495 nm) excitation. This fluorochrome demonstrates little fluorescence under green light excitation (500–570 nm) or UV excitation (330-380 nm).

The number of slides stained will depend upon the species and size of the sections used and the staining container. Using a typical 50 mL Coplin jar, 160 slides (two mouse brain sections per slide) could easily be stained in one afternoon. The entire kit will stain at least 800 such slides or more.

Materials Provided

NisslFluor™ Green RTD™ 10X liquid solution, 40 mL

Detailed protocol

Fluorescent Imaging Settings

NisslFluor™ Green RTD™ labeled neurons are visualized with blue light excitation (like those used for visualizing FITC). A quality FITC-type filter set will work in more instances. A blue-light laser (445-488 nm wavelength) can be used on a laser system.

Storage Instructions:

The stock solution can be stored for up to 6 months after receipt at 2-8°C protected from light. This material contains no preservatives. Use sterile technique when handling and proper laboratory procedures. 1X solutions should be made fresh every day when in use. Diluted 1X solutions are not stable for longer than 12 hours. Keep stock and diluted stains out of light to prolong lifespan. Use best laboratory practices and sterile techniques for best results.

Expiration Date:

Six months after the date of receipt (10X unopened vial). Diluted 1X solutions are not stable for longer than 12 hours. Keep stock and diluted stains out of light to prolong lifespan. Use best laboratory practices and sterile techniques for best results.

We recommend using aseptic techniques to avoid bacterial growth and contamination when handling the reagents. NisslFluor™ Green RTD™ material is shipped ambient and stable at room temperature during transport.

Materials Required but not Supplied

Gelatin-coated or tissue-treated microscope slides (e.g., Superfrost Plus)
Distilled water
ACS grade 100% Ethanol (200 proof) for slide & solution preparation
70% ethanol in distilled water
Xylene liquid
Staining dishes/Coplin jars
Coverslips
Slide warmer
DPX mounting media if required
Convection oven

Solution & Slide Preparations

NisslFluor™ Green RTD™ Working solution:

Prepare the 1X Stain solution by diluting the 10X stock, 1:9, with distilled water. Make only as much 1X stain as you will use in the afternoon. Diluted stain does not keep. Store in the dark. Use a sterile technique for best results.

Ethanol, 70% solution (500 mL):

In a graduated cylinder, add 7 parts absolute Ethanol to 3 parts distilled water and mix gently. **Ethanol and water create an exothermic reaction, so work slowly and carefully.** Store in a sealed bottle at 4°C; remake after 3 months. For example, adding 350 mL ethanol and then 150 mL water to 500 mL total volume provides a 70% ethanol solution.

Preparation of Gelatin-Coated Slides (if using):

The slides are prepared by placing clean slides in a slide rack and in a solution of ethanol for 2 minutes, then in distilled water for 2 minutes. The slides are then transferred to a 1% pig-skin gelatin solution (Sigma: 300 Bloom), heated to 65°C. Drain excess gelatin on a paper towel and transfer to a paraffin-free convection oven overnight at 60° C. After overnight drying, the slides are ready to be used to mount fresh cut or paraffin sections.

Alternatively, sections can be mounted from warm water where .005% gelatin has dissolved and slightly cooled. Such slides with sections should then be dried on a slide warmer at 50°C for at least 2 hours to completely dry and adhere.

Note: Commercially prepared slides can also be used. Our gelatin-coated slides provide a straightforward protocol for those who want to make their own slides with maximum adhesion capabilities if properly prepared and dried. NisslFluor™ Green has successfully been used with SuperFrost Gold slides, and other makes of charged slides should work as well. For best results, it is critical that any section placed on a slide is thoroughly dry. Most failures stem from inadequate drying of the sections to slides. Sections can be mounted onto gelatin-coated or treated slides and dried at 50-60°C on a slide warmer for at least 1 hour or overnight for good adhesion and drying.

Non-buffered polar/aqueous mounting media (optional)

Mix 9 parts molecular biology grade glycerol with 1 part distilled water pH 7.0.

Experimental Methods

Rehydration:

1. Transfer the dried, mounted tissue section slides to a new Coplin jar containing a solution of freshly prepared 70% ethanol (in water) for 2 minutes. Replace after every 20 slides.

2. Transfer slides to a new Coplin jar containing distilled water for 3-5 minutes. Replace water after every 20 slides. Do not let the slides dry out.

3. For free-floating fixed sections, rinse sections with distilled water for 2-3 minutes to remove any contaminating substances.

Staining protocol:

1. Place slides or sections in the prepared 1X fluorescent Nissl staining solution for 10 min, longer if sections are > 25um. Change Nissl 1X solution every 50-100 slides or when the stain weakens.
2. Rinse slides or sections carefully for 3 min in distilled water, and repeat (i.e., do TWO 3-minute washes in water).
3. Differentiate the sections by transferring the slides to a new Coplin jar containing a freshly made 70% ethanol solution for 3 minutes. Replace 70% ethanol solution every 20 slides.

At this point, the slides/sections can be processed for either aqueous or non-polar solvent-based mounting media.

The following processing is used for tissue to be cleared in xylene and mounted with DPX medium.

4a. Transfer slides through 2 three-minute changes of 100% ethanol. Change the ethanol solution every 25-30 slides.

5a. Transfer slides through 2 three min changes of xylene; replace xylene every 100 slides

6a. Coverslip slides with DPX or DePeX non-polar mounting media. View when dry. Keep in the dark when not viewing. Store dried slides at 4°C, dark, with desiccant.

The following processing is used for tissue to be coverslipped with an aqueous mounting medium:

4b. Return slides to DW for 3 min.

5b. Coverslip slides with a non-buffered polar/aqueous mounting media such as 9 parts glycerol mixed with 1 part distilled water. View immediately or store tightly sealed at 4°C. It is a non-permanent mount at this stage and can dry out if not viewed reasonably quickly.

6b. Optional – seal slides along the edges with nail polish to make them more like permanent slides, but this only works if the coverslip edges are dry and free from liquid. Store in the dark, tightly sealed. Slides will dry out over time. Aqueous mounts are not permanent.

For paraffin-embedded tissue section slides:

Slides containing paraffin-embedded sections of brain tissue are de-waxed in xylene 2X 10 minutes each to ensure complete removal of paraffin wax.

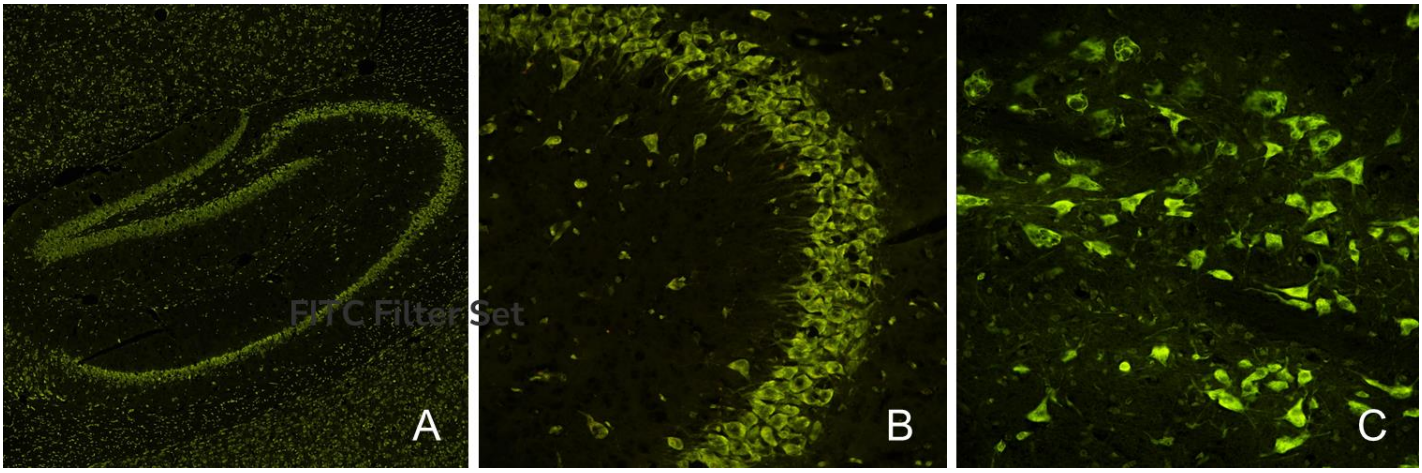
They are immersed in 100% ethanol for 2 x 5 min, then 70% alcohol for 2 minutes, and then rinsed with 2 x 1-minute changes of distilled water. Then, proceed to step “1” of the preceding staining protocol above.

Visualization:

Visualize sections of NisslFluor™ Green by blue light excitation (450-495 nm) using a FITC-type filter block.

NisslFluor™ Green Nissl stain turns neurons' somas, dendrites, cytoplasm, and nuclei fluorescent green under blue light. Nissl is rarely found in axon hillocks or axons.

NisslFluor™ Green will be of high contrast, resolution, and brightness and resistant to fading. The fluorochrome exhibits minimal bleed-through when excited by longer or shorter wavelengths and is moderately resistant to fading.



(A) NisslFluor™ Green, The neurons of the mouse hippocampus appear green following staining with NisslFluor™ Green. Blue light excitation

(B) NisslFluor™ Green labeling can be seen in the cytoplasm and proximal dendrites of neurons within the CA2 region of the mouse hippocampus. Blue light excitation.

(C) A paraffin-embedded sagittal section reveals green NisslFluor™ Green stained magnocellular and parvocellular neurons of the brain stem reticular formation. Blue light excitation.