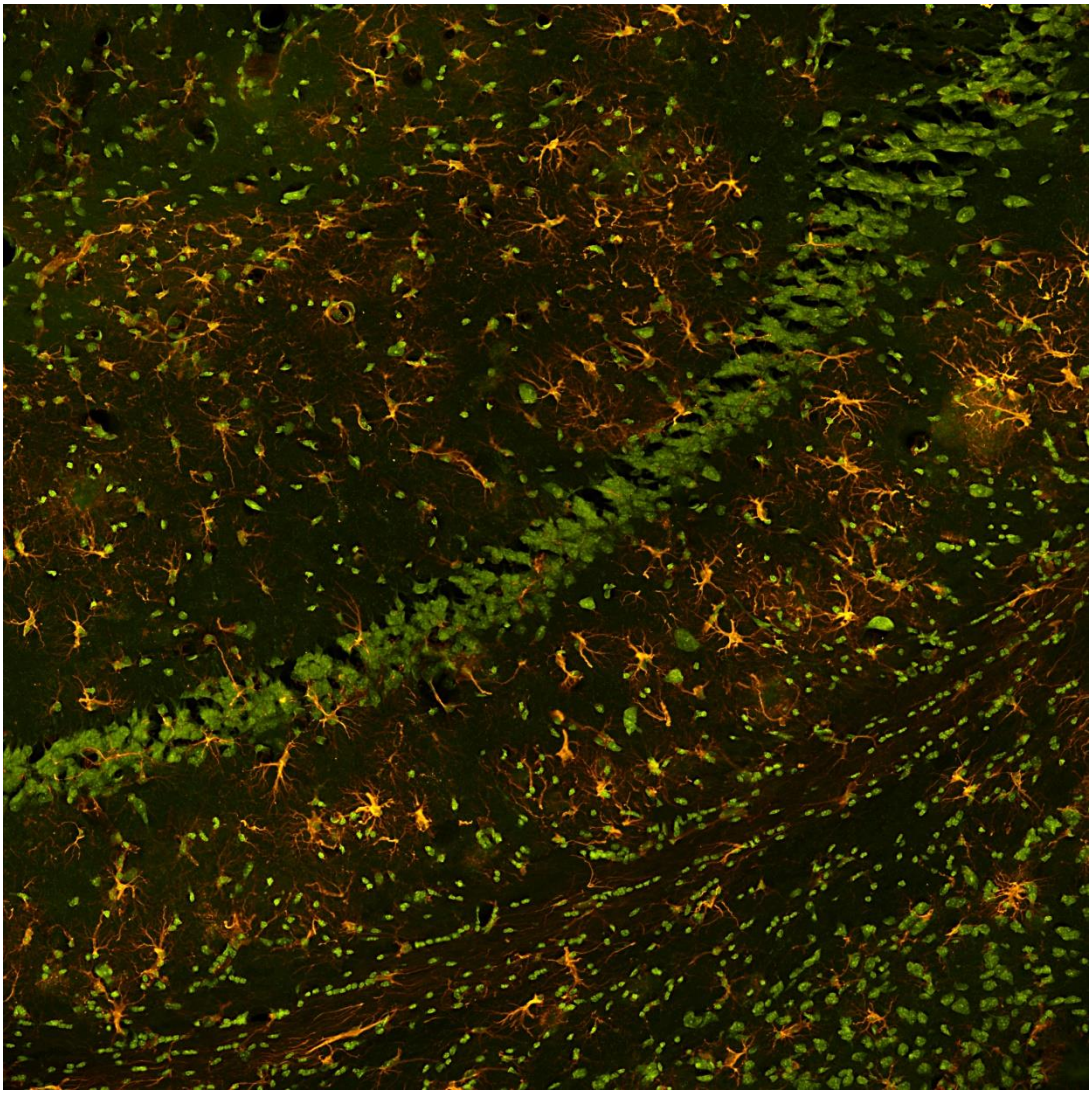
NisslFluor™ Green RTD™

A fluorescent Nissl stain for neurons

Catalog Number: TR-220-NGN



For research use only, not for use in clinical and diagnostic procedures.

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

Differentiating Solution 10X liquid 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. Diluted 1X solutions are stable for 5 days at room temperature, although the best results are usually made with fresh solutions. 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 stable for 5 days at room temperature, although the best results are usually made with fresh solutions. Keep stock and diluted stains out of light to prolong their lifespan. Use best laboratory practices and sterile techniques for the best results.

We recommend using aseptic techniques to avoid bacterial growth and contamination when handling the reagents. NisslFluor™ Red 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)
PBS for diluting the NisslFluor™ dyes {~20 mM, 150 mM NaCl, pH 7.2-7.4}
Distilled water for diluting the differentiation solution
Xylene liquid
Staining dishes/Coplin jars
Coverslips
Slide warmer
DPX mounting media if required
Non-buffered polar/aqueous mounting media aqueous mounting media, (if required, recipe below)
Convection oven (only if making homemade gel-coated slides, procedure below)

Solution & Slide Preparations

NisslFluor™ Green RTD™ Working solution:

Prepare the 1X Stain solution by diluting the 10X stock, 1:9, with PBS. Make only as much 1X stain as you will use within a week. Diluted stain does not keep long.

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. [Note: Complete drying is crucial for proper section adhesion]. After overnight drying, the slides are ready to mount fresh cut or paraffin sections.

Alternatively, fixed tissue 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 55°C for at least 1 hour 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™ Red has successfully been used with SuperFrost Gold slides, and other makes of charged slides should work as well. Any section placed on a slide must be thoroughly dry for best results. 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.

Preparation of Differentiation Solution:

This solution can be made by diluting one part 10X stock differentiating solution with 9 parts distilled water (**do not use PBS**). Change after every 50-80 slides, or the dye is losing its color.

Non-buffered polar/aqueous mounting media (optional)

Mix 9 parts molecular biology grade glycerol with 1 part distilled water pH 6.4-7.0.

Experimental Methods

1. Follow the laboratory's standard IHC procedures to stain free-floating or slide-mounted brain tissue sections with antibodies.

   For the best results, convert antibody-stained free-floating sections to slide-mounted sections. See the above section titled "Preparation of Gelatin-Coated Slides" for instructions.

   If you already use slide-mounted antibody-stained tissue sections, briefly rinse them in distilled water once and place them on a slide warmer at 55°C for at least 1 hour to dry and adhere completely. In our experience, drying will not interfere with fluorescent detection and is required for the best performance of our NisslFluor stains. Alternative staining methods have not been examined.
2. When ready to counterstain, rehydrate slides for 5 min in PBS.
3. Immerse slides in prepared fluorescent Nissl staining solution for 5 minutes. This solution is made by diluting 1 part dye stock solution with 9 parts PBS. Change the Nissl 1X solution every 50-100 slides or when the stain weakens.
4. After staining, rinse through one 5-minute change of distilled water.
5. Differentiate for 1-5 minutes in 1X differentiator solution. This solution can be made by diluting one part 10X stock differentiating solution with 9 parts distilled water (**do not use PBS**). The incubation time will vary depending upon the Nissl Stain used. 1 minute for Nissl Silver, and Nissl Red; 5 minutes for Nissl Green and Nissl Orange.
6. Following differentiation, rinse slides with two 5-minute changes of distilled water.
7. Air dry slides on a slide warmer in the dark, then visualize.
8. Briefly clear dry slides in xylene and coverslip with DPX mounting media (recommended). DPX generally results in a higher resolution and contrast slide, although aqueous mounting media may be needed when using some Alexa-Fluor-labeled secondary antibodies.
9. Alternatively, the slides can be coverslipped with unbuffered aqueous mounting media, such as 9 parts glycerol mixed with 1 part distilled water. View immediately or store tightly sealed at 4°C. At this stage, it is a non-permanent mount and can dry out if not viewed reasonably quickly. Note: Commercial aqueous mounting media has not yet been tested but is expected to be compatible with all NisslFluor™ stains.

   Optional: Seal aqueous mounted slides along the edges with nail polish to make them more like permanent slides. 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 otherwise not permanent.

For paraffin-embedded tissue section slides:

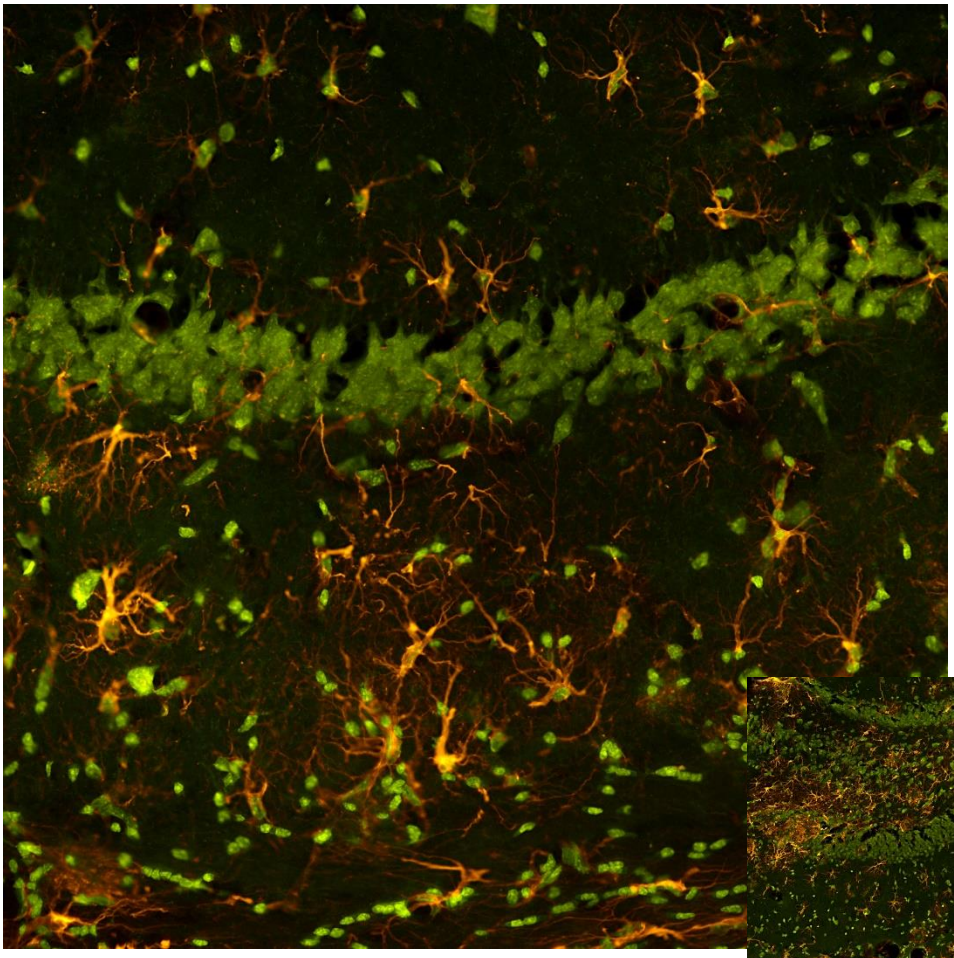
Slides containing paraffin-embedded sections of brain tissue are de-waxed in xylene for 2X 10 minutes each to ensure complete removal of paraffin wax. Typically, they are then rehydrated by immersing them in 100% ethanol for 2 x 5 minutes, then 70% alcohol for 2 minutes, and then rinsed with 2 x 1-minute changes of distilled water. Then, proceed to step "1" of the preceding staining protocol above.

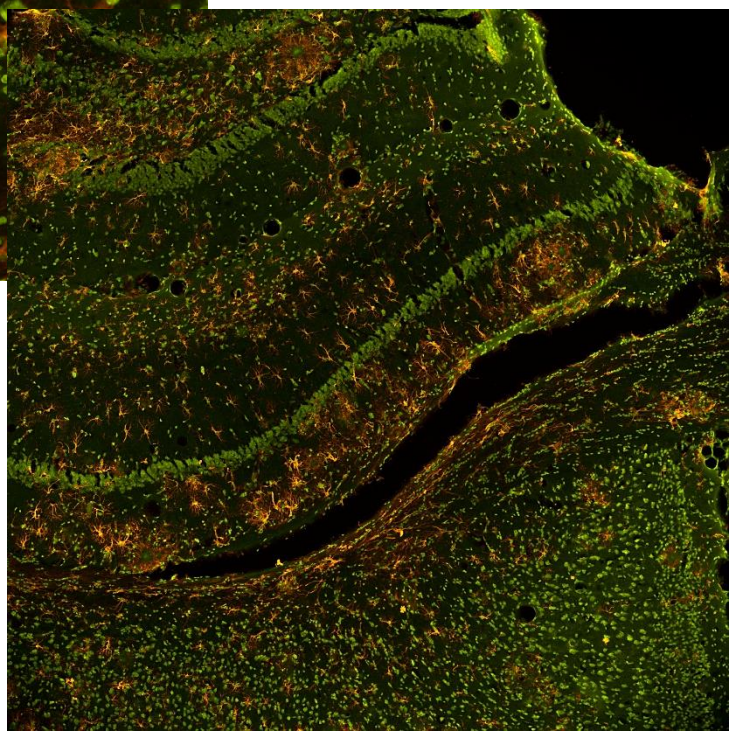
Visualization:

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.

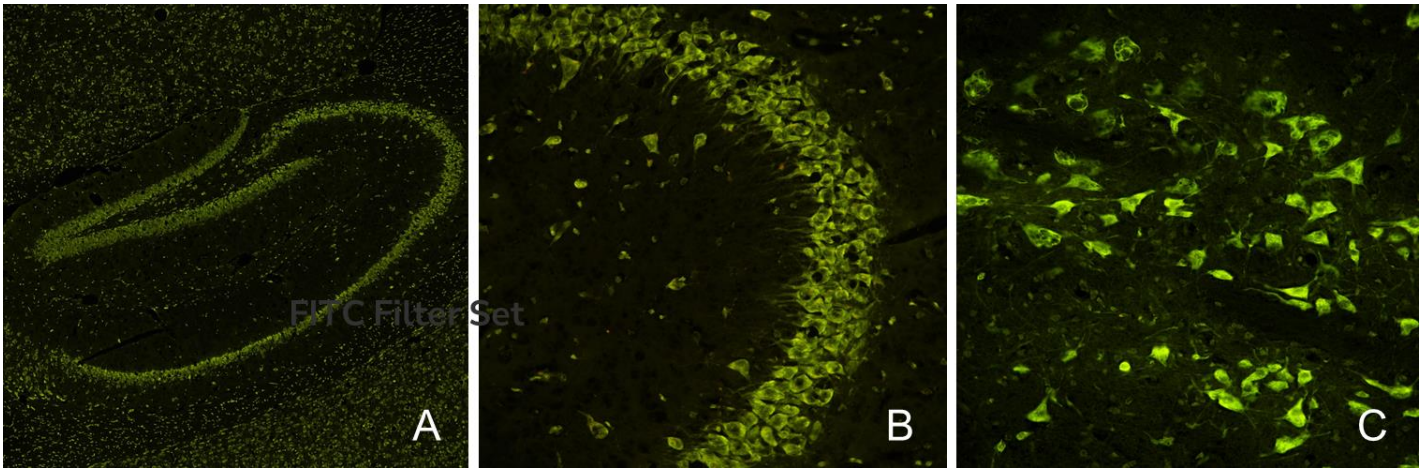
NisslFluor™ Green will be of high contrast, resolution, and brightness and resistant to fading. The fluorochrome exhibits minimal bleed-through when excited by longer or shorter wavelengths and is moderately resistant to fading.





NisslFluor Green™ stained mouse hippocampus tissue (40X) co-labeled with Biosensis chicken anti-GFAP antibody C-1373-50 (above); (10X) (right), and detected using a Tritc-conjugated Rabbit anti-Chicken IgY secondary antibody.

Nissl Stain only images



(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.

Addition information on our NisslFluor ™ Nissl stains:

NisslFluor Name	UV Light	Blue Light	Green Light
NisslFluor Orange™	No fluorescence	Moderate	Bright
NIsslFluor Green ™	No fluorescence	Bright	Moderate
NisslFluor Red ™	Faint	Moderate	Bright
NisslFluor Silver™	Bright	No fluorescence	No fluorescence

The chart above summarizes the spectrofluorometric properties of our versatile Nissl stains. When double labeling, we typically use fluorochromes that fluoresce at different wavelengths to demonstrate the same structure is multiply labeled. However, for counterstaining, dyes that are excited by similar wavelengths but emit different colors may be sufficient, showcasing the adaptability of our stains.

By examining the chart, we can choose a counterstain that would not fluoresce when the primary IHC stain is being imaged. For example, NisslRed™ and Nissl Green™ can be paired with a UV-excited IHC probe such as AMCA. Similarly, Nissl Green 2™ could be combined with a green light-excited orange fluorescing IHC label such as TRITC. Our NisslFluor Silver™ stain is particularly useful as it only fluoresces under UV excitation, making it an ideal counterstain for either red or green IHC labeling.

In addition to their spectrofluorometric properties, the relative contrast and resolution of the labeling are also relevant when selecting the optimal counterstain. The nature of the RNA binding plays a significant role. Nissl Green ™ and Nissl Orange ™, like the conventional cationic bright field Nissl stains, bind to the electronegative phosphate backbone of the RNA molecule. However, Nissl Silver™, Nissl Red™, and Nissl Green 2™ bind to the minor groove between the nucleic acid base pairs, significantly increasing fluorescence upon binding, and are usually brighter and should be considered if the primary antibody also demonstrates strong fluorescence.