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Nissl Staining with Thionin

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We use this protocol and it's working

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Abstract

A "Nissl stain with thionin" is a histological technique used to visualize the cell bodies of neurons in tissue sections, where the primary dye, thionin, binds to the negatively charged components of Nissl bodies (clusters of rough endoplasmic reticulum) within the neuronal cytoplasm. This selective staining allows for clear identification of neurons and their distribution within the tissue. The method is particularly useful for studying neuronal morphology, cell density, and the cytoarchitecture of various brain regions. During the staining process, tissue sections are exposed to a thionin solution, which stains the Nissl bodies, and the excess stain is often removed through a differentiating solution to enhance contrast. Under the microscope, neurons appear as distinct blue or purple structures, making it easy to distinguish them from non-neuronal tissue.

Materials

4% PFA

Paraformaldehyde 4.0g
1x PBS 100ml
**heat to get PFA into solution, then cool to 4C before use*
***don't boil!!*

10xPBS, phosphate buffered saline

To make 1 L of 10X stock:
NaCl 80.0 g
KCl 2.0 g
Na2HPO4 14.4 g
KH2PO4 2.4 g
nano H20 to 1.0 L
**dilute to 1X working concentration*

Thionin Stock

Stock 1.3% thionin:
13 gm Thionin
1000 ml distilled H2O
∗Stir and heat gently for 1 hour. Filter the solution after the dye has dissolved. Store in a stoppered bottle. Use high purity thionin, such as Sigma T3387.

Thionin Buffer

1 M Acetic Acid (HAc):
58.5 ml glacial acetic acid
dilute to 1 liter with distilled water
2. 1 M Sodium Hydroxide (NaOH):
50 gm sodium hydroxide pellets
dissolve to 1 liter with distilled water
∗Mix buffer reagents together and adjust pH before mixing with the thionin stock in the formulas below.

Thionin Stain

80.0 ml 1M HAc
14.4 ml 1M NaOH
305.6 ml Thionin stock

Tissue Fixation

1 Fix tissue in 4% PFA for 15 min

2 Rinse in 1x PBS at least once for 5 min

Staining

3 Rinse in dH2O for 3-5 min

4 Stain in Thionin stain for 2-7 min, depending on number of times stain has previously been used
** Typical schedule: 1-3 uses = 2-3 min; 4-6 uses = 3-4 min; 6-10 uses = 5-7 min*
***Re-make solution after 10 uses*

5 Dehydrate:
70% EtOH with a few drops of Acetic Acid, 15-30 sec, dip twice
70% EtOH, 15-30 sec, dip twice
95% EtOH, 30 sec to several minutes
**This step differentiates the nuclei stain (purple) from the cytoplasm stain (blue)*
***Watch for desired "darkness" of stain (ideally medium blue with a slight hue of purple)*

6 100% EtOH, 30 sec with several swishes back and forth
**Important to completely dehydrate/remove all water from the sections before moving to xylenes*

Mounting Coverslips

7 Clear in xylenes for 3-5 min
**Look for clearing of "fuzzy" or "drippy" (this is moisture trapped in the tissue) appearance before mounting*

8 Drop a dime-seized drop of DPX onto each slide and gently place a 24mmx60mm coverslip on top