

THIONIN (NISSL STAINS) FOR THICK SECTIONS (SOP-22)

Cut sections between 25 and 50 μm thick on sliding microtome.

Mounting step

Float 4 - 6 sections per slide (Fisher SuperFrost Plus or gelatine coated slide) in PBS
Let dry
Place in slide cassette

Adhesion step (in hood; only needed for thick, e.g., Vibratome cut, sections)

2.9 % paraformaldehyde in PBS ~5 minutes
ddH₂O ~1 minute
Let dry

Lipid extraction step (in hood)

dH₂O ~1 minute
50 % EtOH ~3 minutes
70 % EtOH ~3 minutes
95 % EtOH ~3 minutes
95 % EtOH ~3 minutes
100 % EtOH ~3 minutes
100 % EtOH ~3 minutes
xylenes ~5 minutes
xylenes ~5 minutes[§]

Staining Step (in hood)

100 % EtOH ~1 minute
100 % EtOH ~1 minute
95 % EtOH ~1 minute
95 % EtOH ~1 minute
70 % EtOH ~1 minute
50 % EtOH ~1 minute
dH₂O ~1 minute
Thionin, 0.1 % (w/v) in Ac, pH 4.0 2 to 5 minutes
dH₂O ~1 minute
dH₂O ~1 minutes
50 % EtOH ~1 minute
70 % EtOH ~1 minute
95 % EtOH ~1 minute
95 % EtOH ~1 minute*¹
100 % EtOH ~1 minute
100 % EtOH ~1 minute
xylenes ~3 minutes
xylenes ~3 minutes (sections may be held in xylenes)

Coverlip with DPX[™] or Permount[™]

¹ Add 1-2% (v/v) glacial Ac-acid to enhance staining.

THIONIN STAINING STOCK SOLUTIONS

1.0 M Acetic Acid

470 ml ddH₂O
30 ml glacial acetic acid

1.0 M Sodium Hydroxide

250 ml ddH₂O
10 g NaOH

0.1% Thionin, pH 4.0

382 ml ddH₂O
100 ml 1.0 M acetic acid
18 ml 1.0 M NaOH
0.5 g thionin

1. Heat the buffer solution to steaming (60°C), then slowly add the thionin while stirring vigorously.
2. Filter and store the stain in the oven at 57°C. Filter and stain before and after each use.
3. Fresh stain should be made up every 3-6 months.