

THIONIN (NISSL STAINS) FOR THICK SECTIONS

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

Mounting step

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

Adhesion step[#] (only for thick, e.g., Vibratome cut, sections)

4 % paraformaldehyde in PBS ~5 minutes
dH₂O ~1 minute
Let dry

Lipid extraction step[#]

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[#]

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 *
100 % EtOH ~1 minute
100 % EtOH ~1 minute
xylenes ~3 minutes
xylenes ~3 minutes[§]

Coverlip with DPXTM or PermountTM

[#]The steps below must be done in the vented hood.

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

[§]Sections may be held in xylenes

THIONIN STAINING STOCK SOLUTIONS

1.0 M Acetic Acid	470 ml	dH ₂ O
	30 ml	glacial acetic acid

1.0 M Sodium Hydroxide	250 ml	dH ₂ O
	10 gm	NaOH

0.1% Thionin, pH 4.0	382 ml	dH ₂ O
	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.