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
- 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™

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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.