

Nissl Counterstain Protocol

Solutions needed

100% Ethanol (EtOH)	1.22 L
Acetic Acid (CH ₃ COOH)	7 drops
Chloroform (CHCl ₃)	200 mL
HemoDe	200 mL
MilliQ water (H ₂ O)	380 mL
Permunt	a couple drops per stained slide
Thionin Buffer	200 mL
Thionin Stain	200 mL

Materials needed

Absorbent bench liner	Plastic slide racks (for drying slides)
Coverslips	Square glass containers (13)
Glass slide racks (for staining slides)	Transfer pipette

% of ethanol solution	Amount of 100% ethanol per container (mL)	Amount of MilliQ water per container (mL)	# of containers needed
100 %	200	0	2
95 %	190	10	2
70 %	140	60	2
50 %	100	100	1
30 %	60	140	1

Note. Do all steps under the fume hood. Steps are time-sensitive and will move quickly once the protocol starts. Run through the full protocol with one slide as a test before running the rest of the slides – this is to ensure that all steps are correct.

Protocol

Workspace setup

1. Prepare the work area by labeling each square glass container.
 - a. Write each of the following labels on separate containers: Chloroform, 50% EtOH, 30% EtOH, Buffer, Thionin Stain, 70% EtOH, 70% EtOH + Acetic Acid, HemoDe.
 - b. Label two containers each with the following: 100% EtOH, 95% EtOH.
 - c. Order the containers so that they follow the staining protocol.
2. Prepare 200 mL of solution for each corresponding container. Use the table above for making the different concentrations of ethanol. Add 7 drops of acetic acid to one of the 70% EtOH solutions.
 - a. For larger containers, it may be necessary to add more than 200 mL so that the slide will be completely covered when the slide rack is lowered into the liquid.

3. Place slides (with brain sections to be stained) in a glass slide rack that can easily fit inside each square glass container.

Staining

For each step: Submerge the slides, held in a glass slide rack, in the specified solution for the required amount of time. Dry the slides off between each bath by dabbing the slide holder on absorbent bench liner.

1. Delipidizing:
 - a. Chloroform for 40 minutes.
2. Rehydrating:
 - a. 100% EtOH for 1 minute.
 - b. 95% EtOH for 1 minute.
 - c. 70% EtOH for 1 minute.
 - d. 50% EtOH for 1 minute.
 - e. 30% EtOH for 1 minute.
3. Staining:
 - a. Buffer for 1 minute.
 - b. Thionin stain for 3 minutes.
 - c. Buffer for 15 seconds.
4. Dehydrating:
 - a. 30% EtOH for 30 seconds.
 - b. 50% EtOH for 30 seconds.
 - c. 70% EtOH + acetic acid for ~1:45 minutes.
 - Watch the colour of the sections and adjust the time in the bath accordingly. Start checking the colour at the one-minute mark and then keep checking every 15 seconds.
 - d. 95% EtOH for 1 minute x3.
 - e. 100% EtOH for 1 minute x3.
 - f. HemoDe for 4 minutes x2.
 - Leave the slides in the last bath of HemoDe until coverslipping.
5. Coverslipping:
 - a. Pour Permount into a falcon tube and use a transfer pipette to place a few drops on a slide. Lower the coverslip onto the slide at an angle in order to minimize the number of trapped bubbles.
6. Once cover-slipped, lay the slides out on absorbent bench paper and cover with paper towel.