

THIOFLAVINE-S /LIPOFUSCIN AUTOFLUORESCENCE QUENCHING PROTOCOL (1/04)

Stock solutions:

0.05% KMnO₄/PBS

0.2% K₂S₂O₅/0.2% oxalic acid/PBS

40% EtOH/60% PBS

50% EtOH/50% PBS

1. Deparaffinize sections and hydrate to DH₂O
2. If tissue is EtOH fixed, immerse in NBF for 1 hour followed by a 10 minute rinse in tap H₂O. If fixed in NBF go to step 3
3. Immerse in PBS 5 min.
4. Immerse in 0.05% KMnO₄/PBS 20 min.
5. Rinse in PBS 2 X 2 min.
6. Destain in 0.2% K₂S₂O₅/0.2% oxalic acid/PBS until brown color has been removed from sections (approximately 1 min).
7. Rinse in PBS 3 X 2 min.
8. Immerse in freshly made 0.0125% thioflavine-S/40% EtOH/60% PBS 3 min. in dark (use green boat).
9. Differentiate in 50% EtOH/50% PBS for 10-15 min (green boat).
10. Rinse in PBS 3 X 5 min (green boat).
11. Rinse in DH₂O 5 min (green boat).
12. Coverslip with aqueous anti-fade medium (Vectashield) and seal edges with nail polish.