

Thioflavin-S staining protocol

1. **Deparaffinization and hydration of tissue sections:** Place slides in holders and treat with the clearing agent xylene (paraffin solvent) and a series of graded EtOH as follows:

100% xylene – 5 min

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50%/50% xylene/100% EtOH – 3 min

100% EtOH – 3 min

95% EtOH – 3 min

70% EtOH – 3 min

50% EtOH – 3 min

Water – 2 x 3 min

*Note: Change the solns after about 5 uses or when you see the soln is not cleanly running off the slides, indicating too much paraffin in them.

2. Incubate in filtered 1% aqueous Thioflavin-S for 8 minutes at room temperature (filter Thioflavin-S before each use). Note: protect thioflavin-S from light, and protect the stained slides from light as much as possible. Thioflavin-S stain should be stored at 4C.

3. Wash 2x 3 min in 80% ethanol

4. Wash 3 min in 95% ethanol

5. Wash with 3 exchanges of distilled water

6. Coverslip in aqueous mounting media and allow slides to dry in the dark overnight.

8. Next day, seal coverslip with clear nail polish.

9. Analyze slides within the next few days-weeks because the staining will fade with time. Store the slides in the dark at 4C.