



# KIDDRC Histology Services Protocols

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## Thionin Staining for frozen tissues

### Stock Solutions:

1. 1.0 M Sodium Acetate Solution: 16.4g Sodium Acetate (anhydrous) dissolved in 200 ml distilled H<sub>2</sub>O.
2. 1.0 M Glacial Acetic Acid: add 12.2 ml Glacial Acetic Acid to 200 ml distilled H<sub>2</sub>O.
3. 0.5% Thionin Solution: 0.5 gm thionin in 100 ml distilled H<sub>2</sub>O.

The above solutions are stock solutions. They have a fairly long shelf life. Thionin is hard to dissolve. Use a glass stirring rod or a stir bar. **Do not use a metal rod to stir the thionin.** Filter the thionin Solution.

### Thionin Staining Solution:

1. 180 ml distilled H<sub>2</sub>O
2. 9 ml Sodium Acetate Solution
3. 21 ml Acetic Acid Solution

This is the buffer Solution.  
**Adjust pH to 4.3...then add**

4. 18 ml 0.5% thionin Solution.

### Staining Protocol for Fixed Tissue:

1. Distilled H<sub>2</sub>O.....3-5 min
2. Thionin Stain.....20 min
3. Distilled H<sub>2</sub>O.....3-5 min
4. Distilled H<sub>2</sub>O.....3-5 min
5. 70% Ethanol.....5 min
6. 70% Ethanol.....3 min
7. 95% Ethanol.....3 min
8. ABS Ethanol.....3 min
9. Xylene.....3 min
10. Xylene.....5 min

Coverslip slides wet with permount or microkit.

### Staining Protocol for Fresh-Frozen Sections:

1. 70% Ethanol.....5 min
2. 50% Ethanol.....1 min
3. Distilled H<sub>2</sub>O....1 min
4. Thionin Stain...10 min
5. Distilled H<sub>2</sub>O...30 sec
6. Distilled H<sub>2</sub>O...30 sec
7. 50% Ethanol.....30 sec
8. 95% Ethanol.....30 sec
9. ABS Ethanol.....30 sec
10. Xylene.....30 sec
11. Xylene.....3 min

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