

STOCK SOLUTIONS

Bouin's Solution – see recipe in “Fixatives”. Add glacial acetic acid (to 5%) just before use.

Alcoholic Hematoxylin Stock (Solution A)

| <u>Component</u> | <u>Cat. No.</u> | <u>Amount</u> |
|------------------|-----------------|---------------|
| • Hematoxylin | Sigma H9627 | 5 g |
| • 95% Ethanol | | 500 mL |

Dissolve over gentle heat – do not boil. Label with date and initials. Store at room temperature up to 1 year.

10% Ferric Chloride Stock (Solution B)

| <u>Component</u> | <u>Cat. No.</u> | <u>Amount</u> |
|---------------------------------------|-----------------|---------------|
| • Ferric chloride (Iron III chloride) | Sigma F7134 | 5.8 g |
| • Milli-Pure water | | 495 ml |
| • Glacial Acetic Acid | | 5 ml |

Dissolve ferric chloride in 50 ml water, then add remaining water to 990 ml. Add acetic acid last. Store at room temperature for up to 1 year.

Acid Fuchsin, 1% Aqueous Stock Solution

Dissolve 1 g acid fuchsin (Sigma #857408) in 100 mL Milli-Pure water. Label and store at room temperature.

Biebrich Scarlet, 1% Aqueous Stock Solution

Dissolve 10 g biebrich scarlet (aka Ponceau BS, Sigma #B6008) in 1000 mL Milli-Pure water. Label and store at room temperature.

Aniline Blue Stock Solution

| <u>Component</u> | <u>To make 50 mL</u> | <u>To make 75 mL</u> | <u>To make 250 mL</u> |
|----------------------------------|----------------------|----------------------|-----------------------|
| • Aniline Blue (CAS #66687-07-8) | 1.25 g | 1.875 g | 6.25 g |
| • Glacial acetic acid | 1.0 mL | 1.5 mL | 5.0 mL |
| • DI water | 50 mL | 75 mL | 250 mL |

Store at room temperature for up to 1 year.

Phosphomolybdic-Phosphotungstic Acid Solution

| <u>Component</u> | <u>To make 50 mL</u> | <u>To make 75 mL</u> | <u>To make 250 mL</u> |
|--|----------------------|----------------------|-----------------------|
| • Phosphomolybdic acid (Sigma #221856) | 0.625 g | 0.938 g | 3.125 g |
| • Phosphotungstic acid (Sigma #P4006) | 0.625 g | 0.938 g | 3.125 g |
| • DI Water | 50 mL | 75 mL | 250 mL |

Store at room temperature for up to 1 year.

WORKING SOLUTIONS**1% Acetic Acid Solution**

| <u>Component</u> | <u>To make 50 mL</u> | <u>To make 75 mL</u> | <u>To make 250 mL</u> |
|-----------------------|----------------------|----------------------|-----------------------|
| • Glacial acetic acid | 0.5 mL | 0.75 mL | 2.5 mL |
| • Distilled Water | 49.5 mL | 74.25 mL | 247.5 mL |

Make fresh just before use.

Weigert's Hematoxylin – Working Solution

Masson's Trichrome Stain

Nuclei, Cytoplasm, Erythrocytes, Keratin, Muscle Fibers, Collagen, Mucus

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| <u>Component</u> | <u>To make 50 mL</u> | <u>To make 75 mL</u> | <u>To make 250 mL</u> |
|--|----------------------|----------------------|-----------------------|
| • Solution A – Alcoholic Hematoxylin Stock | 25 mL | 37.5 mL | 125 mL |
| • Solution B – 10% Ferric Chloride Stock | 25 mL | 37.5 mL | 125 mL |

Prepare just before use. Discard in "Hematoxylin Waste" container.

Biebrich Scarlet-Acid Fuchsin Solution – Working Solution

| <u>Component</u> | <u>To make 50 mL</u> | <u>To make 75 mL</u> | <u>To make 250 mL</u> |
|------------------------------|----------------------|----------------------|-----------------------|
| • Biebrich scarlet, 1% stock | 44.5 mL | 66.75 mL | 222.5 ml |
| • Acid fuchsin, 1% stock | 5.0 mL | 7.5 mL | 25 ml |
| • Glacial Acetic Acid | 0.5 mL | 0.75 mL | 2.5ml |

Prepare just before use. Can be used for a maximum of two runs if within 24 hours.

Aniline Blue Working Solution

| <u>Component</u> | | | |
|-------------------------------|--------|--------|--------|
| • Aniline Blue Stock Solution | 50 ml | 75 ml | 250 ml |
| • Glacial acetic acid | 1.0 ml | 1.5 ml | 5.0 ml |

Add glacial acetic acid just before using.

PROCEDURE:*****Always run a control slide with every stain!*****

1. Deparaffinize and re-hydrate tissues to PBS*
2. Distilled water* 1 min.
3. Mordant in Bouin's – in fume hood, covered Overnight
4. Running tap water rinse – until yellow color clears 1 min.
5. Distilled water rinse 1 min.
6. Weigert's Hematoxylin working solution* 12 min.
 - a. *Discard after use.*
7. Running tap water rinse 10 min.
8. Distilled water rinse 1 min.
9. Biebrich scarlet-acid fuchsin solution 1 min.
 - a. *Solution may be used twice only, then discarded.*
10. Distilled water rinse 1 quick dip only
11. Phosphomolybdic-phosphotungstic acid solution 30 min.
 - a. *Discard after use.*
12. Aniline blue solution* 10 min.
 - a. *Solution may be used twice only, then discarded.*
13. Distilled water rinse 1 quick dip only
14. 1% Acetic acid solution 4 min.
 - a. *Discard after use.*
15. 95% Ethanol 2 x 1 min.
16. 100% Ethanol 2 x 1 min.
17. Formula 83 3 x 1 min.
18. Mount with Permount

***MODIFICATIONS FOR CRYO-SECTIONS:**

1. Fix sections in **10%formalin** (not Zinc) x 30 minutes.
2. Replace steps 1, 2 with 3 x 3 min. rinses in distilled water.
3. Reduce time in hematoxylin and aniline blue to 7 minutes each.

RESULTS: Cytoplasm, keratin, muscle fibers, Erythrocytes – red

Nuclei – black

Collagen and mucus – blue

REFERENCES:

Carson, Freida L., Histology, A Self-Instructional Text. 1990. Pp. 142-144, and Plate 8-4, p. 267.

As cited by the American Ukrainian Medical Project at:

<http://aump.org/resources/documents/histo/en/MassonsTrichrome.pdf>