

Usage procedure for:

## Trichrome Stain Kit, Masson, Aniline Blue

Avantik Kit Item #: RS4570-125 | RS4570-250 | RS4570-500



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### Solutions Provided in Stain Kit:

Bouin's Solution  
Iron Hematoxylin, Solution A  
Iron Hematoxylin, Solution B  
Biebrich Scarlet Acid Fuchsin Solution  
Phosphomolybdic Phosphotungstic Acid  
Aniline Blue Stain  
Acetic Acid, 1%

Working solution preparation (*stable for one month*):

### Working Iron Hematoxylin tSolution

Iron Hematoxylin, Solution A:	25mL
Iron Hematoxylin, Solution B:	25mL

### Conventional Procedure

1. Deparaffinize and hydrate sections through alcohol to distilled water.
2. Rinse section thoroughly in distilled water.
3. Place formalin fixed solutions in **Bouin's Solution** for **1 hour** at 56°C.
4. Allow slides to cool and thoroughly rinse sections in running tap water until the yellow from the Bouin's Solution disappears.
5. Rinse slides in distilled water.
6. Stain in **ClearView™ Iron Hematoxylin Working Solution** for **10 minutes**.
7. Rinse well using distilled water.
8. Stain in **Biebrich Scarlet-Acid Fuchsin Solution** for **2 minutes**.
9. Rinse slides in distilled water.
10. Place sections in **Phosphomolybdic-Phosphotungstic Acid Solution** for **10-15 minutes**.
11. Stain in **Aniline Blue Stain** for **5 minutes**.
12. Rinse well using distilled water.
13. Place slides in **Acetic Acid, 1%** for **3-5 minutes**.
14. Dehydrate slides in two changes each 95% and 100% Alcohol.
15. Clear and mount with appropriate mounting medium.

### Results:

Nuclei:	Black
Collagen, Mucin:	Blue
Cytoplasm, Keratin, Muscle/Intercellular Fibers:	Red

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**NOTE:** It is important to remember each microwave is different. Below are guidelines using 50mL of each solution in vented plastic (or loosely capped) Coplin jars, heated in a 500 Watt microwave at full power. Be sure not to heat any solution to boiling. Care should be applied the first time using this kit with your microwave. Note any variation in time to be used during further applications. If you are currently using a similar stain kit with the same components listed below and have already established correct working times for your laboratory, as a general rule, your times may be used with this kit. Care should still be applied the first time using this kit and validated using a control slides.

### Microwave Procedure

1. Deparaffinize and hydrate sections through alcohol to distilled water.
2. Rinse section thoroughly in distilled water.
3. Place formalin fixed sections in **Bouin's Solution**, heat for **30 seconds** (DO NOT BOIL) and *incubate* for **2-3 minutes**. **Note:** Due to the explosive nature of Picric Acid when dry, we recommend placing the Coplin jar in a partially closed plastic bag to trap any accidental boilover or spill of the Bouin's Solution. This step can be omitted if tissues were originally fixed in Bouin's Solution.
4. Allow slides to cool and thoroughly rinse sections in running tap water until the yellow from the Bouin's Solution disappears, approx. 5 minutes.
5. Place slides in **ClearView™ Iron Hematoxylin Working Solution**, *heat* for **30 seconds** and *incubate* for **30-60 seconds**. **Note:** Keep solution away from any source of direct heat and/or sparks.
6. Rinse slides in running tap water for 1 minute.

Note: We recommend checking the control for desired staining intensity. If more staining time is needed, place back into heated solution and repeat Step 6.

7. Blue sections in bluing solution of choice for 30-60 seconds.
8. Rinse slides in running tap water for 1 minute.
9. Place slides in **Biebrich Scarlet-Acid Fuchsin Solution**, *heat* for **30 seconds** and *incubate* for **1-2 minutes**.
10. Rinse slides in distilled water.
11. Place slides in **Phosphomolybdic-Phosphotungstic Acid Solution**, *heat* for **30 seconds** and *incubate* for **1-2 minutes**.
12. Rinse slides in distilled water.
13. Place slides in **Aniline Blue Stain**, *heat* for **30 seconds** and *incubate* for **1-2 minutes**.
14. Rinse well using distilled water.
15. Place slides in ROOM TEMPERATURE **Acetic Acid, 1%** for **3-5 minutes**.
16. Dehydrate slides in two changes each 95% and 100% Alcohol.
17. Clear and mount with appropriate mounting medium.

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### Results:

Nuclei:	Dark Blue to Black
Collagen, Mucin:	Blue
Cytoplasm, Keratin, Muscle/Intercellular Fibers:	Red

### Kit Components and Related Control Slides:

Avantik Item Numbers:

**Bouin's Solution:** RS4396-250 (250 mL) | RS4396-500 (500 mL)

**Iron Hematoxylin, Solution A:** RS4418-250 (250 mL) | RS4418-500 (500 mL)

**Iron Hematoxylin, Solution B:** RS4419-250 (250 mL) | RS4419-500 (500 mL)

**Biebrich Scarlet Acid Fuchsin Solution:** RS4395-250 (250 mL) | RS4395-500 (500 mL)

**Phosphomolybdic Phosphotungstic Acid:** RS4436-250 (250 mL) | RS4436-500 (500 mL)

**Aniline Blue Stain:** RS4394-250 (250 mL) | RS4394-500 (500 mL)

**Acetic Acid, 1%:** RS4383-250 (250 mL) | RS4383-500 (500 mL)

**Trichrome Control Slides:** SL6219-A (Pack/12) | SL6219-B (Pack/25) | SL6219-D (Pack/100)

**Trichrome Pos/Neg Control Slides:** SL6380-A (Pack/12) | SL6380-B (Pack/25) | SL6380-D (Pack/100)