American MasterTech
scientific laboratory supplies
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MASSON'S TRICHROME STAIN KIT PROCEDURE

Item# CUKTMTRLT ***(Revised 03/7/18)***

KIT COMPONENTS INCLUDED:

- **2 pints BOUIN'S FLUID**
- **1 liter ANILINE BLUE STAIN**
- **2 pints WEIGERT'S HEMATOXYLIN "A"**
- **1 liter PHOSPHOMOLYBDIC/ PHOSPHOTUNGSTIC ACID**
- **1 liter BIEBRICH SCARLET ACID FUCHSIN**
- **1 liter 1% ACETIC ACID**
- **2 pints WEIGERT'S HEMATOXYLIN "B"**

PRINCIPLE: This kit demonstrates collagen and muscle.

SPECIMEN: Any well fixed paraffin embedded tissue cut at 4 to 6 microns.

QUALITY CONTROL: American Master*Tech Scientific Recommended Control Slide: Heart, CSH0725P; Skeletal Muscle, CSS0725P; Uterus, CSU0325P

PROCEDURE:

1. Deparaffinize slide with Xylene or Xylene Substitute and hydrate through alcohols to Tap water.
2. Mordant sections in **BOUIN'S FLUID** for 1 hour at 56°C or for enhanced results, overnight at room temperature.
3. Rinse slide in running Tap water until tissue is colorless.
4. Place slide in **WEIGERT'S HEMATOXYLIN** for 5 minutes. (**Mix equal parts Weigert's "A" & "B" just before use.**)
5. Rinse slide thoroughly in running Tap water.
6. **Place in **BIEBRICH SCARLET- ACID FUCHSIN** for 15 minutes.
7. Rinse slide in Distilled water.
8. Place slide in **PHOSPHOMOLYBDIC / PHOSPHOTUNGSTIC ACID** for 10 to 15 minutes.
9. Without rinsing, place slide in **ANILINE BLUE STAIN** for 5 to 10 minutes.
10. Rinse slide in Distilled water.
11. Place slide in **1% ACETIC ACID** for 3 to 5 minutes.
12. Dehydrate slide through 2 changes of 95% Reagent Alcohol followed by 2 changes of Absolute Alcohol.
13. Clear slide through 3 changes of Xylene or Xylene Substitute.
14. Coverslip using a permanent mounting media.

SPECIAL PROCEDURE

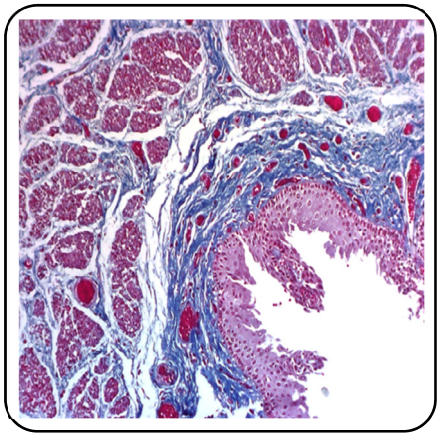
** For Central Nervous System (C.N.S.) Sections, after **STEP 5** use the following **STEPS**:

6. Place slide in **BIEBRICH SCARLET-ACID FUCHSIN** for 1 to 2 minutes.
7. Rinse slide in Distilled water.
8. Place slide in **PHOSPHOMOLYBDIC-PHOSPHOTUNGSTIC ACID** for 10 to 30 minutes.
9. Without rinsing, place slide in **ANILINE BLUE STAIN** for 15 to 20 minutes.
10. Continue procedure at **Step 10**.

COMPONENT NOTE: Low-hazard **Master*Tech Bouin's 2000™ Tissue Fixative** can be used at **Step 2**; mordant sections for 1.5 hours at 56º C or overnight at room temperature.

RESULTS:

Cytoplasm, Keratin, Muscle, Intercellular Fiber: **RED**
Collagen, Mucus: **BLUE**
Nuclei: **BLACK**



Human Esophagus

REFERENCE: Sheehan DC Hrapchak BB: Theory and Practice of Histotechnology; 1980, 190.
A.F.I.P. Laboratory Methods in Histotechnology: 1992, 132 - 133.

ALTERNATE PROCEDURE NOTE: Microwave Masson's Trichrome Stain Kit Procedure on opposite side.

MICROWAVE MASSON'S TRICHROME STAIN KIT PROCEDURE

Revised 04/28/11

KIT COMPONENTS INCLUDED:		
2 pints	**BOUIN'S FLUID**	**1 liter BIEBRICH SCARLET ACID FUCHSIN**
1 liter	**ANILINE BLUE STAIN**	**1 liter 1% ACETIC ACID**
2 pints	**WEIGERT'S HEMATOXYLIN "A"**	**2 pints WEIGERT'S HEMATOXYLIN "B"**
1 liter	**PHOSPHOMOLYBDIC/ PHOSPHOTUNGSTIC ACID**	

PRINCIPLE: This kit demonstrates collagen and muscle.

SPECIMEN: Any well fixed paraffin embedded tissue cut at 4 to 6 microns.

QUALITY CONTROL: American Master*Tech Scientific Recommended Control Slide: Heart, CSH0725P; Skeletal Muscle, CSS0725P; Uterus, CSU0325P

TECHNICAL SPECIFICATIONS: These instructions were developed using a 500 Watt microwave oven, at full power, using 25 ml of each solution in a plastic Screw Top-Slide Jar. Adjust heating times when using a larger volume of solution. (*Plastic Screw Top Slide-Jars are available from American Master*Tech Scientific.)*

PROCEDURE:

1. Deparaffinize slide with Xylene or Xylene Substitute and hydrate through alcohols.
2. Rinse slide in running Tap water.
3. Place slide in **BOUIN'S FLUID**, heat for 20 seconds *(Do not boil!)* and incubate for 2 minutes.
4. Rinse slide in running Tap water for 5 minutes.
5. Place slide in **WEIGERT'S HEMATOXYLIN (Mix equal parts Weigert's "A" & "B" just before use),** heat for 20 seconds and incubate for 30 seconds.
6. Rinse slide in running Tap water for 1 minute, then blue section for 30 seconds.
7. Rinse slide thoroughly in running Tap water.
8. Place slide in **BIEBRICH SCARLET-ACID FUCHSIN**, heat for 20 seconds and incubate for 2 minutes.
9. Rinse slide in Distilled water.
10. Place slide in **PHOSPHOMOLYBDIC/PHOSPHOTUNGSTIC ACID**, heat for 20 seconds and incubate for 1 minute.
11. Rinse slide in Distilled water.
12. Place slide in **ANILINE BLUE STAIN**, heat for 20 seconds and incubate for 45 to 90 seconds.
13. Rinse slide in Distilled water.
14. Place slide in room temperature **1% ACETIC ACID** for 3 to 5 minutes..
15. Dehydrate slide through 2 changes of 95% Reagent Alcohol followed by 2 changes of Absolute Alcohol.
16. Clear slide through 3 changes of Xylene or Xylene Substitute.
17. Coverslip using a permanent mounting media.

RESULTS:

Cytoplasm, Keratin, Muscle, Intercellular Fiber: **RED**
Collagen, Mucus: **BLUE**
Nuclei: **DARK BLUE TO BLACK**

REFERENCE: Sheehan DC Hrapchak BB: Theory and Practice of Histotechnology; 1980, 190.
A.F.I.P. Laboratory Methods in Histotechnology: 1992, 132 - 133.