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MASSON'S TRICHROME STAIN KIT PROCEDURE

BP2916 100 Test

KIT COMPONENTS INCLUDED:

100ml. BOUIN'S FLUID
100ml. ANILINE BLUE STAIN
100ml. WEIGERT'S HEMATOXYLIN "A"
100ml. PHOSPHOMOLYBDIC/ PHOSPHOTUNGSTIC ACID
100ml. BIEBRICH SCARLET-ACID
100ml. 1% ACETIC ACID
100ml. WEIGERT'S HEMATOXYLIN "B"

PRINCIPLE: This kit demonstrates collagen and muscle.

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

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℃, 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 (Mix equal parts Weigert's "A" & "B" just before use**), stain for 5 min.
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. 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:

1. Place slide in **Biebrich Scarlet-Acid Fuchsin** for 1 to 2 minutes.
2. Rinse slide in Distilled water.
3. Place slide in **Phosphomolybdic-Phosphotungstic Acid** for 10 to 30 minutes.
4. Place slide in **Aniline Blue Stain** for 15 to 20 minutes.
5. Continue procedure at **Step #10.**

RESULTS:

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

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

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MICROWAVE MASSON'S TRICHROME STAIN KIT PROCEDURE

KIT COMPONENTS INCLUDED:

100ml. BOUIN'S FLUID
100ml. ANILINE BLUE STAIN
100ml. WEIGERT'S HEMATOXYLIN "A"
100ml. PHOSPHOMOLYBDIC/ PHOSPHOTUNGSTIC ACID
100ml. BIEBRICH SCARLET-ACID FUCHSIN
100ml. 1% ACETIC ACID
100ml. WEIGERT'S HEMATOXYLIN "B"

PRINCIPLE: This kit demonstrates collagen and muscle.

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

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.

PROCEDURE:

1. Deparaffinize slide with Xylene or Xylene Substitute and hydrate through alcohols to Tap water.
2. Place slide in **Bouin's Fluid**, heat for 20 seconds *(Do not boil!)* and incubate for 2 minutes.
3. Rinse slide in running Tap water for 5 minutes.
4. 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.
5. Rinse slide in running Tap water for 1 minute, then blue section for 30 seconds.
6. Rinse slide thoroughly in running Tap water.
7. Place slide in **Biebrich Scarlet-Acid Fuchsin**, heat for 20 seconds and incubate for 2 minutes.
8. Rinse slide in Distilled water.
9. Place slide in **Phosphomolybdic/Phosphotungstic Acid**, heat for 20 seconds and incubate for 1 minute.
10. Rinse slide in Distilled water.
11. Place slide in **Aniline Blue Stain**, heat for 20 seconds and incubate for 45 to 90 seconds.
12. Rinse slide in Distilled water.
13. Place slide in room temperature 1% Acetic Acid for 3 to 5 minutes..
14. Dehydrate slide through 2 changes of 95% Reagent Alcohol, followed by 2 changes of Absolute Alcohol.
15. Clear slide through 3 changes of Xylene or Xylene Substitute.
16. Coverslip using a permanent mounting media.

RESULTS:

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

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