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Fite's Staining Kit

(Cat# K1425-125; Store at RT)

I. Introduction:

The Fite's Staining Kit (for Leprosy and Nocardia) is intended for use in the histological visualization of mycobacterium Lepra bacillus (leprosy) and Nocardia. This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Lepra bacillus: Red; Nocardia: Red; Background: Blue

II. Application:

- Histological applications
- For *in vitro* diagnostic use

III. Sample Type:

- Formalin-fixed, paraffin-embedded (5 microns) or frozen sections.
- Control Tissue: Any well-fixed paraffin embedded Nocardia or Lepra bacillus infected tissue.

IV. Kit Contents:

Components	K1425-125	Part Number	Storage Temperature
Xylene-Peanut Oil Solution	125 ml	K1425-125-1	RT
Carbol Fuchsin Solution	125 ml	K1425-125-2	RT
Acid Alcohol Solution (0.5%)	500 ml	K1425-125-3	RT
Methylene Blue Solution	125 ml	K1425-125-4	RT

V. User Supplied Reagents and Equipment:

- Distilled water
- Coplin jars
- Xylene/Xylene Substitute
- Synthetic resin

VI. Shipment and Storage:

All the reagents are shipped and stored at RT.

VII. Reagent Preparation:

- Do not use if reagents become cloudy.
- Do not use past expiration date.
- Use caution when handling reagents.
- Non-Sterile.

VIII. <u>Lepra bacillus Procedure (Standard):</u>

1. Deparaffinize sections in 2 changes of Xylene-Peanut Oil Solution for 12 minutes each.
2. Air dry slide for 15 min "without" removing oil film covering tissue section. Remaining film prevents de-staining of Lepra bacillus during differentiation.
3. Rinse slide in several changes of distilled water.
4. Incubate slide in Carbol Fuchsin Solution for 15 min.
5. Rinse slide in several changes of distilled water.
6. Differentiate section in Acid Alcohol Solution (1%) until background is pink.
7. Rinse slide in distilled water and check by microscope for correct differentiation.
8. Rinse in running tap water for 1 minute followed by 1 rinse in distilled water.
9. Dip slide 2-3 times in Methylene Blue Solution.
10. Dip slide quickly in distilled water and check by microscope for correct staining.
11. Air dry slide at room temperature.
12. Dip slide several times in Xylene or Xylene Substitute.
13. Mount in synthetic resin.

<u>Nocardia Procedure:</u>

Preparation of Reagents Prior to Beginning:

Prepare Diluted Acid Alcohol Solution by mixing 25 ml of Acid Alcohol Solution (1%) with 25 ml of Distilled Water.

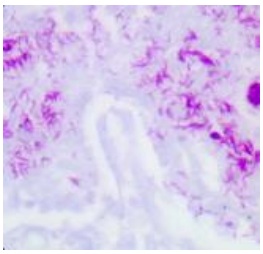
<u>Procedure:</u>

1. Deparaffinize sections in 2 changes of Xylene-Peanut Oil Solution for 12 minutes each.
2. Air dry slide for 15 minutes "without" removing oil film covering tissue section. Remaining film prevents de-staining of Lepra bacillus during differentiation.
3. Rinse slide in several changes of distilled water.
4. Incubate slide in Carbol Fuchsin Solution for 15 minutes.
5. Rinse slide in several changes of distilled water.
6. Dip slide 2-3 times in Diluted Acid Alcohol Solution.
7. Rinse slide in distilled water and check by microscope for correct differentiation. Avoid decolorizing the Nocardia organism.
8. Rinse in running tap water for 1 minute followed by 1 rinse in distilled water.
9. Dip slide 2-3 times in Methylene Blue Solution.
10. Dip slide quickly in distilled water and check by microscope for correct staining.
11. Air dry slide at room temperature.
12. Dip slide several times in Xylene or Xylene Substitute.
13. Mount in synthetic resin.

IX. Troubleshooting:

- Acid-fastness of the leprosy organisms is enhanced when the waxy capsule is protected by the mixture of xylene/peanut oil and the avoidance of dehydrating solutions.
- It is important to blot well; residual oil may produce staining artifact.
- If over-stained with methylene blue, organisms may be masked. Check microscopically before air drying. If over-stained, remove methylene blue with Acid Alcohol 1%; rinse thoroughly; repeat Methylene Blue Solution step with a shorter timing.
- If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
- A small percentage of Nocardia sp. organisms may resist taking the red stain and remain blue due to the growth phase of the individual organism.

X. Data:



Staining using Fite's Staining Kit