



VitroView™ Reticulum Stain Kit

SKU No. VB-3018

Introduction

Reticular fibers are very thin and delicately woven strands of type III collagen. These fibers are not visible with the H & E stain but they react very well with ammoniacal and methenamine silver stains. This kit is designed for the detection of reticular fibers in histological tissue specimens.

Kit Components

VB-3018-1 10% Silver Nitrate Solution	-----40 ml
VB-3018-2 Concentrated Ammonium Hydroxide	-----20 ml
VB-3018-3 3% Sodium Hydroxide Solution	-----20 ml
VB-3018-4 Potassium Permanganate Solution	-----100 ml
VB-3018-5 Potassium Metabisulfite Solution	-----100 ml
VB-3018-6 Ferric Ammonium Sulfate Solution	-----100 ml
VB-3018-7 10% Formaldehyde Solution	-----100 ml
VB-3018-8 0.1% Gold Chloride Solution	-----100 ml
VB-3018-9 5% Sodium Thiosulfate Solution	-----100 ml
VB-3018-10 Nuclear-Fast Red Solution	-----100 ml

Storage

Store 10% Silver Nitrate Solution and 0.1% Gold Chloride Solution at 2-8°C. Others at room temperature

Things to do before starting

To make Working Ammoniacal Silver Solution: Using chemically cleaned glassware or virgin plastic ware add 10ml of Silver Nitrate Solution to 17 ml distilled water and mix thoroughly. Then add 1 ml of Concentrated Ammonium Hydroxide while mixing; the solution will turn light brown, and then become colorless. Add 10 ml of 3% Sodium Hydroxide Solution and mix thoroughly; solution should be colorless; if not, add Concentrated Ammonium Hydroxide drop by drop until no precipitate remains. To complete solution, add distilled water to obtain a total volume of 60 ml and mix thoroughly.

Protocol

1. Deparaffinize in Xylene I for 6 minutes and II for 6 minutes.
2. Rehydrate.
 - a. Ethanol 100% (2 minutes)×2
 - b. Ethanol 95% (2 minutes)×2
 - c. Ethanol 70% (2 minutes)×1
3. Rinse in distilled water (5 minutes).
4. Potassium permanganate solution, 5 minutes.
5. Wash in distilled water
6. Differentiate in Potassium Metabisulfite Solution until clear.
7. Wash in distilled water.
8. Apply Ferric Ammonium Sulfate Solution for 10 minutes.
9. Wash in running tap water, rinse in distilled water, 3 changes
10. Apply Working Ammoniacal Silver Solution, 7 dips, and shakes excess solution off slides.
11. Distilled water, 2 changes, 3 quick dips each.
12. 10% Formaldehyde Solution until gray black, 30 seconds.
13. Wash in distilled water.

14. 0.1% Gold chloride, 1 minute.
15. Rinse in distilled water.
16. 5% Sodium Thiosulfate Solution, 1 minute.
17. Wash in tap water.
18. Nuclear-fast Red Solution, 5 minutes.
19. Wash in running tap water for 5 min.
20. Dehydrate with 2 changes of 95% Ethanol and 2 changes of 100% Ethanol (2 minute per change).
21. Clear with 3 changes of xylene (5 minutes per change) and coverslip with Permount or other suitable organic mounting medium.

Control Tissue: Liver, kidney, lymph node, spleen

Results:

- Reticular fibers-----black
- Nuclei-----red

Note

This product is intended for research purposes only. This product is **not** intended to be used for therapeutic or diagnostic purposes in humans or animals.

Precautions

Handle with care in chemical hood. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.