# Usage procedure for:

## Reticulum Stain Kit

### Avantik Kit Item #: RS4566-125 | RS4566-250 | RS4566-500



19 Chapin Rd - Building C Pine Brook, NJ 07058

- t: 800.783.9424
- e: info@avantik-us.com

avantik-us.com

### **Solutions Provided in Stain Kit:**

Potassium Permanganate, 1%
Oxalic Acid, 1%
Ferric Ammonium Sulfate, 2.5%
Silver Nitrate, 10%
Sodium Hydroxide, 3%
Ammonium Hydroxide, 28%
Formalin Solution, 10% (unbuffered)
Gold Chloride, 0.1%
Sodium Thiosulfate, 5%
Nuclear Fast Red

Working solution preparation (prepare just before use):

### **Ammoniacal Silver Solution**

Place 5mL of Silver Nitrate, 10% in an appropriate container. Add Ammonium Hydroxide, 28% drop by drop until the precipitate that forms is dissolved. Be sure not to add any excess Ammonium Hydroxide. Add 5mL Sodium Hydroxide, 3%. Cautiously dissolve the precipitate that forms once again by adding Ammonium Hydroxide, 28% drop by drop until only a faint cloudiness remains. Dilute the resulting solution to 50mL with distilled water. Use immediately and discard after each use.

#### **Conventional Procedure**

- 1. Deparaffinize and hydrate sections through alcohol to distilled water.
- 2. Oxidize sections in Potassium Permanganagte, 1% for 5 minutes.
- 3. Wash slides in running tap water for 2 minutes. **Note:** Due to differences in tap water quality, several changes of Deionized Water may be used in place of running tap water rinses if tap water has proven suspect in staining procedures in the past for your lab.
- 4. Place slides in Oxalic Acid. 1% for 2 minutes or until sections are colorless.
- 5. Wash slides in running tap water for 2 minutes.
- 6. Place slides in Ferric Ammonium Sulfate, 2.5% for 15 minutes.
- 7. Rinse slides thoroughly in several changes of deionized water.
- 8. Place slides in Ammoniacal Silver Solution for 2 minutes.
- 9. Rinse slides thoroughly in several changes of deionized water.
- 10. Place slides in the Formalin Solution, 10% for 2 minutes.
- 11. Wash slides in running tap water for 3 minutes.
- 12. Tone sections in Gold Chloride, 0.1% for Rinse slides thoroughly in several changes of deionized water
- 5-10 minutes. Avoid excess toning in Gold Chloride, which will result in an undesirable red tone.

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- 13. Wash slides in running tap water for 2 minutes.
- 14. Place slides in Sodium Thiosulfate, 5% for 1 minute.
- 15. Wash slides in running tap water for 2 minutes.
- 16. Counterstain (if desired) in Nuclear Fast Red for 3-5 minutes.
- 17. Thoroughly rise slides in distilled water.
- 18. Dehydrate in 2-3 changes of 95% alcohol followed by 2-3 changes of 100% alcohol.
- 19. Clear and mount with appropriate mounting medium.

### **Results:**

Reticulum Fibers: Black

Background: Pink to Red (If counter staned with Nuclear Fast Red)

### **Kit Components and Related Control Slides:**

Avantik Item Numbers:

**Potassium Permanganate**, **1%**: RS4439-250 (250 mL) | RS4439-500 (500 mL)

Oxalic Acid, 1%: RS4433-250 (250 mL) | RS4433-500 (500 mL)

Ferric Ammonium Sulfate, 2.5%: RS4403-250 (250 mL) | RS4403-500 (500 mL)

**Silver Nitrate**, **10%**: RS4447-250 (250 mL) | RS4447-500 (500 mL)

**Sodium Hydroxide**, **3%**: RS4453-250 (250 mL) | RS4453-500 (500 mL)

**Ammonium Hydroxide**, **28%**: RS4391-250 (250 mL) | RS4391-500 (500 mL)

Formalin Solution, 10% (unbuffered): RS4406-250 (250 mL) | RS4406-500 (500 mL)

Gold Chloride, 0.1%: RS4411-250 (250 mL) | RS4411-500 (500 mL)

**Sodium Thiosulfate**, **5%**: RS4455-250 (250 mL) | RS4455-500 (500 mL)

Nuclear Fast Red: RS4429-250 (250 mL) | RS4429-500 (500 mL)

**Reticulum Control Slides:** SL6381-A (Pack/12) | SL6381-B (Pack-25) | SL681-D (Pack/100)