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**TECHNICAL DATA SHEET 269**



# Multiple Stain Solution (MSS)

## INTRODUCTION:

Polysciences, Inc. Multiple Stain Solution (MSS) is a replacement for the former Paragon Multiple Stain (PMS) Solution. Multiple Stain Solution is a combination of toluidine blue and basic fuchsin in ethanol. The stain differentiates acidophilic and basophilic structures in tissue samples, smears, and cell preparations. Staining is a single step procedure that results in a hematoxylin and eosin-like appearance. Use Multiple Stain Solution on frozen sections, both unfixed and fixed, epoxy resins, JB-4®, JB-4 Plus®, GMA, and MMA sections.

Utilize Multiple Stain Solution at full strength or dilute if longer staining times or more control of the staining procedure is required. A 3% to 5% dilution of Multiple Stain Solution in deionized water can used in any of the procedures listed below, but provides much better control for plastic sections. The staining times for a 3% to 5% dilution will be approximately 1 to 3 minutes depending on the intensity of the stain required. Dilutions of Multiple Stain Solution are not as stable as the full strength solution. Prepare diluted stain in small amounts and use within one week.

## PROCEDURE FOR FROZEN UNIFIXED SECTIIONS

Staining of unfixed sections or cells is considered semi permanent:

1. Section frozen tissue at 4µ to 6µ in a cryostat. Sections are picked up on a glass slide.
2. Allow the slide to dry briefly in the cryostat and remove to an area with running water.
3. Apply Multiple Stain Solution directly on the slide with a pipette, covering the tissue completely. Stain for 15 to 30 seconds depending on the intensity required.
4. Rinse the slide carefully with gently running tap water.
5. Drain off excess water and wipe the back of the slide dry.
6. The slide can be examined under the microscope with or without a coverslip.

## PROCEDURE FOR FROZEN FIXED SECTIONS

Staining of fixed frozen sections, cells, or smears is considered permanent. Begin the procedure at Step 2 for preparations of cells and smears. If the slide is cover-slipped with Aqua-Poly/Mount Mounting Media immediately, it will not require dehydration and clearing. Skip Step 6 and coverslip immediately. *Do not allow the slide to dry between steps.*

1. Section frozen tissue at 4µ to 6µ in a cryostat. Sections are picked up on a glass slide.
2. Place slides directly in fixative, either alcoholic formalin or Neutral buffered formalin, for 30 seconds to one minute or as required by the laboratory protocol.
3. Rinse the slide carefully with gently running tap water.
4. Apply Multiple Stain Solution directly on the slide with a pipette, covering the tissue completely. The slide may also be submerged in a Coplin jar or placed in a staining dish with full strength Multiple Stain Solution. Stain for 15 to 30 seconds depending on the intensity required.
5. Rinse the slide carefully with gently running tap water.
6. Dehydrate very quickly through two 95% ethanol baths, two absolute alcohol baths, and two xylene baths. Coverslip with Poly-Mount® Mounting Media or other permanent mounting media.
   Note: *The section can be differentiated if the stain is too dark by using longer dips in the dehydration steps.*

## PROCEDURE FOR JB-4, JB-4 PLUS, GMA, OR MMA SECTIONS

Complete the dehydration steps very quickly. Do not allow slides with plastic sections to sit in any of the alcohols or xylene. Over exposure will cause the sections to begin to lift or curl. These sections also lose staining intensity very quickly.

1. Cut JB-4, GMA, or MMA sections at 1µ to 4µ and pick up with tweezers. Place on a waterbath to allow the sections to move around releasing the hydrophobic tendencies. If the JB-4, JB-4 Plus, or GMA sections are placed on a drop of water they may become wrinkled during movement and unusable.
2. Remove excess water from the slide by draining and gently soaking up the water from the section edge or placing on a hot plate at approximately 55° to 60°C until completely dry (15 to 30 minutes). Strips of filter paper can be used to absorb excess water around the section edge.
3. Apply Multiple Stain Solution directly on the slide with a pipette covering the tissue completely. Stain for 15 to 30 seconds depending on the intensity required. The slide may also be submerged in a 3% Multiple Stain Solution bath for 1 to 3 minutes.
4. Rinse the slides carefully in gently running tap water.
5. Dehydrate very quickly through two 95% ethanol baths, two absolute ethanol baths, and two xylene baths. Coverslip with Poly-Mount® Mounting media or other permanent mounting media as quickly as possible.

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## PROCEDURE FOR STAINING EPOXY SECTIONS:

1. Epoxy resins can be treated exactly like the JB-4, JB-4 Plus, GMA, and MMA sections through sectioning. See Step 1 in Procedure for JB-4, GMA, or MMA sections.
2. Float sections on a drop or two of water on a glass slide.
3. Place the glass slide on a hot plate at approximately 60°C to dry. Care should be taken to assure the water is completely evaporated prior to adding the stain.
4. Rinse the slide carefully in gently running tap water.
5. Apply Multiple Stain Solution directly on the slide with a pipette covering the tissue completely. Stain for 15 to 30 seconds depending on the intensity required. Dilutions of Multiple Stain Solution are not recommended for epoxy sections.
6. Rinse the slide carefully in gently running tap water. Allow to air dry or dry on the hot plate.
7. If staining is too intense, dehydrate very quickly through two 95% ethanol baths, two absolute alcohol baths, and two xylene baths.
8. Coverslip with Poly-Mount® Mounting Media or other permanent mounting media.

**NOTE:** Sodium borate can be used to enhance Multiple Stain Solution for Osmium Tetroxide ($OsO_4$) post-fixed tissues and routine epoxy sections. Add a pinch of sodium borate to the stain solution on the slide or premix 10ml of Multiple Stain Solution with 0.02 gram of sodium borate weekly.

## RESULTS:

Nuclei - Blue
Cytoplasm - Various shades of pink which identify different tissue and cell components

## STORAGE INSTRUCTIONS:

The solutions should be tightly capped in the original bottle. Store at room temperature. The solution can filtered before use.

## CAUTION:

Flammable! This solution should be kept away from flames, sparks and other sources of ignition.

## ORDERING INFORMATION:

| Cat. # | Description | Size |
|---|---|---|
| 08824 | Multiple Stain Solution | 100mL |
| | | 500mL |
| | | 1000mL |
| 18606 | Aqua-Poly/Mount Mounting Media | 20mL |
| | | 100mL |
| | | 5 x 20mL |
| 08381 | Poly-Mount® Mounting Media | 120mL |
| | | 940mL |
| 09860 | Alcohol Reagent (100%), Histology Grade | 1 gal |
| 08389 | Xylene, Histology Grade | 1 gal |
| 07441 | Microscope Slides, Plain | 1 box |
| 21911 | Microscope Slides, Frosted | 1 box |
| 22247 | Poly-L-Lysine Coated Microscope Slides | 1 box |
| 24216 | Tissue Tack Microscope Slides | 1 box |

## TO ORDER

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