

Basement Membrane Control Slides – Technical Memo

CONTROL SLIDES:	Part 4055A	Part 4055B
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining kidney.

Fixation: Formalin 10%, Phosphate Buffered (Part 1090).

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: Jones Basement Membrane quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.

Storage: 15-30°C in a light deprived and humidity controlled environment.

Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Jones Basement Membrane Stain Kit	Part 9167A	Individual Stain Solution
Solution A: Methenamine 3%, Aqueous	250 ml	Part 12239
Solution B: Silver Nitrate 5%, Aqueous	50 ml	Part 13805
Solution C: Sodium Borate 5%, Aqueous	50 ml	Part 13826
Solution D: Periodic Acid 0.5%, Aqueous	250 ml	Part 13308
Solution E: Gold Chloride 0.25%, Aqueous	250 ml	Part 11287
Solution F: Sodium Thiosulfate 2.5%, Aqueous	250 ml	Part 13889
Solution G: Light Green SF Yellowish Stain 0.1%, Aqueous	250 ml	Part 12203

APPLICATION:

Newcomer Supply Basement Membrane Control Slides are for the positive histochemical staining of basement membranes in tissue sections.

PRESTAINING PREPARATION:

- Heat dry sections in oven according to your laboratory protocol.
- All glassware/plasticware must be acid cleaned prior to use.
 - See Procedure Notes #1 and #2 (page 2).
- Prepare Silver-Methenamine Working Solution and mix well:
 - Solution A: Methenamine 3%, Aqueous 40 ml
 - Solution B: Silver Nitrate 5%, Aqueous 2 ml
 - Solution C: Sodium Borate 5%, Aqueous 4 ml
- Preheat Silver-Methenamine Working Solution to 45°-60°C in a water bath 20-30 minutes before use.
 - Maintain solution between 45°-60°C to minimize precipitate.
 - Do not preheat solution if using Microwave Modification.

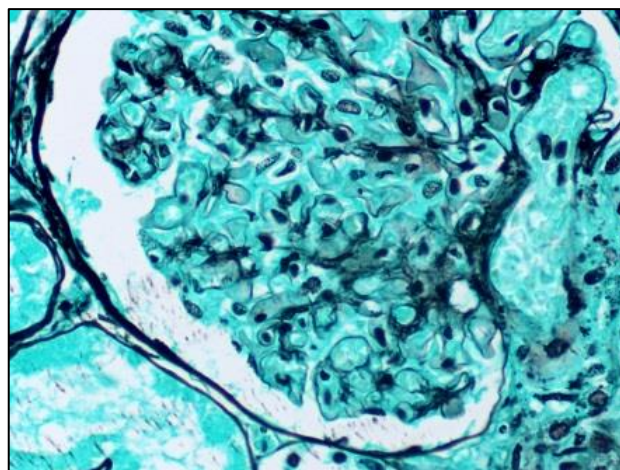
- Rinse in three changes of distilled water.
- Tone in Solution E: Gold Chloride 0.25%, Aqueous for 1 minute.
- Rinse well in three changes of distilled water.
- Place in Solution F: Sodium Thiosulfate 2.5%, Aqueous; 2 minutes.
- Wash in tap water for 5 minutes; rinse in distilled water.
- Counterstain in Solution G: Light Green SF Yellowish Stain 0.1%, Aqueous for 1 minute.
- Quickly rinse slides in two changes of distilled water.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Kidney glomerular basement membranes	Black
Intra-glomerular deposits	Black
Reticular Fibers	Black
Nuclei	Outlined in black
Background	Light Green

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - See Procedure Notes #3 and #4 (page 2).
- Place in Solution D: Periodic Acid 0.5%, Aqueous for 15 minutes.
- Wash in tap water for 5 minutes; rinse in distilled water.
- Incubate slides in preheated Silver-Methenamine Working Solution (Step #4) at 45°-60°C oven or at room temperature for 12-18 minutes until sections appear paper-bag brown.
- Periodically remove control; rinse in warm distilled water, check microscopically for adequate silver impregnation. Basement membranes should be dark brown. If tissue structures are not sufficiently dark, return slides to warm silver solution. Recheck at 2-3 minute intervals until desired intensity is achieved.
 - Staining at room temperature will require longer incubation.
- Microwave Modification:** See Procedure Note #5 (page 2).
 - Place slides in a plastic Coplin jar with prepared Silver-Methenamine Working Solution (Step #3) and microwave at 70°C for 3 minutes.
 - Check microscopically for adequate development.
 - If additional incubation is required, return slides to heated silver solution and recheck at regular intervals.



PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with silver solutions to prevent precipitation of silver salts. No metals of any kind should come in contact with silver solutions. Only glass thermometers should be used.
3. Drain slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during procedure.
5. The suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Jones, David B. "Nephrotic Glomerulonephritis," *American Journal of Pathology* 33.2 (1957): 313–329.
2. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 97-99.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 187-188.
4. Modifications developed by Newcomer Supply Laboratory.