Hexamine silver staining solution (Jones brilliant green staining solution)

Product Code: 246909

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Product Introduction

Jones' light green staining solution is also known as hexamine silver staining solution or basement membrane hexamine silver staining solution. It is a classic staining method for demonstrating basement membranes.

After tissue is oxidized with periodic acid, basement membrane mucopolysaccharides expose aldehyde groups. These aldehyde groups reduce hexamine silver to black metallic silver. Gold chloride converts metallic silver into more stable metallic gold and helps make the background clearer. Thiosulfate solution fixes the staining and removes unreduced silver ions.

Hexamine silver staining clearly demonstrates basement membranes. It is most commonly used for renal lesions and is often used to observe morphological changes in the glomerular capillary basement membrane during inflammatory injury, such as rupture, proliferation, and folding.

This product is used for qualitative histological staining to identify capillary basement membranes.

Package Contents

Pack Size	Code	Component	Volume / Amount
20T	246909.1	Periodic Acid Solution	40 mL
20T	246909.2	Hexamethylenetetramine Powder	2 × 0.18 g
20T	246909.3	Silver Solution	2 × 0.3 mL
20T	246909.4	Borax Solution	2 × 20 mL
20T	246909.5	Gold Chloride Solution	40 mL
20T	246909.6	Thiosulfate Solution	40 mL
20T	246909.7	Light Green Staining Solution	40 mL
20T	246909.m	Instructions	1 copy

Quality Standards and Safety Information

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Periodic Acid Solution	—	—
Hexamethylenetetramine Powder	—	—
Silver Solution	—	—
Borax Solution	—	—
Gold Chloride Solution	—	—
Thiosulfate Solution	—	—
Light Green Staining Solution	—	—

Transportation and Storage

Transportation	Transport on dry ice.
Storage	Store at -20°C. Shelf life: 18 months.

Instructions for Use

1. Preparation of Hexamine Silver Working Solution

1. Take 1 tube of hexamethylenetetramine powder and 1 tube of silver solution.
2. Completely dissolve both components in 1 bottle of borax solution.
3. Mix vigorously for 2 minutes. The borax solution may be repeatedly added to the tube containing hexamethylenetetramine powder and silver solution, then finally collected back into the borax solution bottle.
4. Pour the fully dissolved working solution into a clean staining jar for use.

> If a few transparent or black particles are visible to the naked eye, this is normal and filtration is not required.

Store the prepared hexamine silver working solution at 4°C protected from light. The prepared working solution is valid for 1 month.

2. Staining Procedure

1. Dewax sections routinely to water.
2. Place sections in periodic acid solution and oxidize for 15 minutes. Rinse with running water for 3–6 minutes, then rinse briefly with distilled water.
3. Preheat the hexamine silver working solution in a 62°C water bath for about 15 minutes.
4. Place the sections into the hexamine silver working solution preheated to 62°C and incubate at constant temperature for 30–60 minutes, until a tobacco-yellow or black reaction is seen on the sections against a yellow-brown background.
5. Remove the sections and rinse with distilled water.
6. Examine microscopically, using the appearance of black silver deposits on the glomerular capillary basement membrane as the criterion. If the staining is not dark enough, rinse with distilled water and return the sections to the hexamine silver working solution to continue the reaction.
7. Rinse with distilled water for 2 minutes.
8. Tone with gold chloride solution for 1–2 minutes, then rinse with distilled water for 1–2 minutes.
9. Treat with thiosulfate solution for 1–2 minutes, then rinse with running water for 1–2 minutes.
10. Counterstain with brilliant green staining solution for 1–2 minutes.
11. Dehydrate routinely, clear routinely with xylene or dewaxing and clearing solution for paraffin immersion, mount with neutral balsam resin, and examine microscopically.

> The working solution must not come into contact with metal ions. Ensure that the water bath temperature reaches 62°C. To keep the working solution temperature constant at 62°C, make sure there is sufficient water in the water bath; generally, the water level should not be lower than two-thirds of the height of the staining jar.

65°C paraffin immersion: 2–3 hours.

3. Staining Results

Staining Site	Color
Glomerular capsule basement membrane	Black
Renal capillary glomerular basement membrane	Black
Fungi	Black
Elastic fibers	Black
Reticular fibers	Black
Pneumocystis carinii cyst wall	Brownish black

Precautions

1. If the prepared hexamine silver working solution cannot be used promptly, store it protected from light in a sealed container at 4°C in a refrigerator and use it within 1 month.
2. It is recommended to measure the temperature of the hexamine silver working solution with a thermometer until it reaches 62°C. If the working solution temperature is below 62°C, extend the preheating time. The water bath should contain sufficient water, generally at a height no less than two-thirds of the staining tank height.
3. If no water bath is available, an oven may be used instead. Place the working solution in a 65°C oven for 2 hours, but the effect is not as good as with a water bath.

4. This method is progressive silver impregnation. The renal tubular basement membrane generally stains earlier than the renal vascular basement membrane, but the renal vascular basement membrane should be used as the observation standard. The glomerular basement membrane should appear black.
5. When staining fungi and Pneumocystis carinii, microscopic control is required. If staining becomes too deep, it may be confused with other cell nuclei. It is recommended to remove the slide for observation when a black reaction is seen, then observe once about every 5 minutes until the morphology of fungi or Pneumocystis carinii cysts is clearly discernible.
6. Containers used for this staining solution should be pre-soaked in cleaning solution. If no cleaning solution is available, use 0.1M hydrochloric acid. Rinse containers thoroughly before use. After use, rinse them with cleaning solution.
7. This product is for in vitro diagnostic use only and should be used by professionals for result interpretation.
8. Read the instructions carefully before use and take appropriate personal hygiene and protective measures. Use within the validity period.
9. After use, waste should be disposed of according to hospital or environmental protection department requirements.
10. Sample requirements: tissue sections should be fully fixed and deparaffinized. For fungi, section thickness should be 5 μm. For basement membrane, section thickness should be 1–4 μm.
11. Product performance indicators: the pH value of the borax solution at 25°C ± 1°C is 8.5–9.5. The color of the light green solution should be green.
12. It is recommended that opened staining solution within its shelf life be used within 6 months. After each use, tighten the bottle cap promptly to avoid evaporation or deterioration.