

ones' Method for Kidney

Catalog #: 26396-Series

Fixation:

10% Buffered Neutral Formalin (#15740) or Russell's Zenker (#64123-05).

Sections:

Cut paraffin at 2µm sections.

Staining Procedures:

1. Deparaffinize and hydrate slides to distilled water.
2. Oxidize in Periodic Acid, 0.5% (#26396-01) for 11 minutes. Wash well in chloride-free water.
3. Prepare Methenamine Silver solution by mixing:
 - a. 42.5 ml Methenamine 3% (#26396-02), 2.5 ml Silver Nitrate, 5% (#26396-02) and 12.0 ml Borate Buffer, pH 8.2 (#26396-04)
4. Place slides in the solution and the entire jar in a water bath at 70°C for approx. 60 – 75 minutes. Check under microscope when slides appear medium brown microscopically. Every 10 minutes, once the medium brown color has been established, rinse slide in 70°C chloride-free water and check under microscope. Rinse again in hot water and return to hot staining solution. As the staining time approaches the end point, check the slides, as above, every 1 – 2 minutes. The entire procedure must be performed quickly to prevent an uneven staining of the tissue. The slides should exhibit a brownish-yellow background, intense black reticulum fibers, and black basement membranes. If the slides become oversaturated, i.e. too black, de-stain in a dilute Potassium Ferricyanide Solution, 0.5% (#26396-11) for one or two dips.
5. Rinse well in distilled water. Tone in Gold Chloride, 0.2% (#26396-05) for 1 minute. If sections are over-toned, place in Sodium Metabisulfite, 3% (#26396-12) for 1 – 2 minutes. Rinse well in distilled water.
6. Place in Sodium Thiosulfate, 3% (#26396-09) for 1- 2 minutes. Wash in running tap water, 10 minutes. Rinse well in distilled water.
7. Stain in Harris' Hematoxylin (#26396-06) containing 2 – 4 ml of Glacial Acetic Acid per 100 ml for 5 -15 minutes. Wash in water.
8. Differentiate in Acid Alcohol, 1% (#26396-13) until the sections turned red.
9. Blue section in Ammonia Water, 0.3% (#26396-14). Wash thoroughly.
10. Counter-stain in Eosin Y, 1%, Alcoholic Solution (#26396-07)
11. Dehydrate in 95% and 100% alcohol, and clear in Xylene, 3 changes each. Mount.

Stain Results:

Basement membranes, reticulum fibers solution	Black
Nuclei	Blue
Cytoplasm, collagen, connective tissue	Pink-Orange

References:

Jones, D.B., Amer. J. Path. 27:99 (1951).

AFIP Manual of Histological Staining Methods, 3rd ed., Ed. L. Luna: NY, McGraw-Hill Publ., c. 1968, p.97.