

Instructions For Use GEM-IFU

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18-25°C

Glycogen, Elastin, Mucin Stain Kit

Description and Principle

This stain kit utilizes a sulfation procedure and Toluidine Blue O to give specific metachromatic staining of Glycogen, Elastin, and Mucin (acidic and neutral) in formalin-fixed paraffin-embedded sections. Similar methods have previously been studied and referenced as Aldehyde Bisulfite Toluidine (ABT) and Permanganate Bisulfite Toluidine (PBT) staining^{1, 2}. Glycogen may be visualized with a contrasting background as an improvement upon PAS staining.

Tissue carbohydrates are oxidized to aldehydes by the action of periodic acid or potassium permanganate. Sulfation Reagent adds strongly acidic groups to aldehydes which facilitates selective metachromatic staining by Toluidine Blue Solution, pH 1.0. Stabilization Reagent preserves metachromasia staining normally lost to dehydration and mounting

Expected Results

Glycogen, Elastin, Mucin:	Metachromatic pink to purple	
Background:	Blue	
Kit Contents		Storage
1. Periodic Acid Solution (1%)		2-8°C
2. Potassium Permanganate S	olution (1%)	18-25°C
3. Sulfation Reagent		18-25°C
4. Toluidine Blue Solution, pH	1.0	18-25°C

<u>Suggested Controls</u> (not provided) Liver, Muscle, GI tract, Skin, etc.

Uses/Limitations

5. Stabilization Reagent

For Research Use Only. Do not use if reagents become cloudy or precipitate Do not use past expiration date. Use caution when handling reagents. Non-Sterile Intended for FFPE sections cut at 5-10µm. This procedure has not been optimized for frozen sections. Frozen sections may require protocol modification.

Storage

Mixed storage conditions. Store according to individual label instructions.

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure:

1. Deparaffinize sections if necessary and hydrate to distilled water.

 Apply either Periodic Acid Solution (1%) for 5-15 minutes or Potassium Permanganate Solution for 10 minutes based on desired staining.
 Note: Potassium Permanganate is critical to stain elastin.
 Periodic Acid provides optimal glycogen and mucin staining.

3. Rinse in distilled water.

4. Apply Sulfation Reagent for 30 minutes.



A) Glycogen staining at 20X on Human Liver. Incubation – 15 mins in PAQ, 5 mins in TBO.
B) Elastin staining at 20X on Human Kidney. Incubation – 10 mins in PPE, 15min in TBO.
C) Mucin staining at 20X on Human Stomach. Incubation – 5 mins in PAQ, 5 mins in TBO 5. Quickly rinse in 2 changes distilled water. Rinsing beyond this amount may reduce staining.

6. Apply Toluidine Blue Solution, pH 1.0 for 5-15 minutes based on desired staining intensity. *Note: We prefer 5-10 minutes for mucin and glycogen and 15 minutes for elastin.*

7. Rinse in distilled water.

8. Apply Stabilization Reagent for 2 minutes.

9. Rinse in **Absolute Alcohol.** Do not rinse in water, doing so may invalidate results.

10. Dehydrate in Absolute Alcohol.

11. Clear, and mount in **solvent-based** synthetic resin. Not compatible with aqueous-based mounts.

Staining Notes:

Specificity and intensity of certain histological elements may be changed by the oxidation and sulfation used (alternative reagents not supplied). The other following tissue elements may be specifically highlighted with the following procedural modifications.

Glomerular Basement Membrane in Kidney: Oxidize with Periodic Acid 5% for 15 mins. Use provided Sulfation Reagent.

Pneumocystis jiroveci and other fungi: Use provided oxidizer. Sulfate with mixture of concentrated sulfuric and acetic acids for 10 minutes.

> 1 part sulfuric acid 3 parts glacial acetic acid Instructions: Carefully pour acetic acid followed by sulfuric acid into a clean staining jar and mix well.

References

1. McManus, J.F.A. and Mowry, R. 1955. Staining Methods and Histologic and Histochemical. Grocott, pp 194-197.

2. Sale, G.E. 1978. Rapid Methenamine Silver Stain. Arch Path Lab Med, 1978, 102, pp 351-352.

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6. Procop, G.W. et al. 2004. Detection of Pneumocystis jiroveci in Respiratory Specimens by Four Staining Methods. Journal of Clinical Microbiology. July 2004, Vol. 42, No. 7, pp 3333-3335.

7. Bryan, R.A., Jiang, Z., Howell, R.C., Morgenstern, A., Bruchertseifer, F., Casadevall, A., Dadachova, E. Radioimmunotherapy is More Effective than Antifungal Treatment in Experimental Cryptococcal Infection. Journal of Infectious Disease, August 15, 2010. Vol. 202(4): pages 633-637.

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