

## Alcian Blue Stain 1%, pH 1.0 - Technical Memo

### SOLUTION:

Alcian Blue Stain 1%, pH 1.0 Aqueous	250 ml Part 1005A	500 ml Part 1005B
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### Additionally Needed:

Hydrochloric Acid 0.1N, Aqueous	Part 1207
Nuclear Fast Red Stain, Kernechtrot	Part 1255
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842

**For storage requirements and expiration date refer to individual bottle labels.**

### APPLICATION:

Newcomer Supply Alcian Blue Stain 1%, pH 1.0 Aqueous is for the specific staining of sulfated acid epithelial mucins in the Alcian Blue Stain 1%, pH 1.0 procedure.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)  
**Technique:** Paraffin sections cut at 5 microns  
**Solutions:** All solutions manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

### STAINING PROCEDURE:

1. 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.
  - a. See procedure note #1.
2. Rinse sections briefly in Hydrochloric Acid 0.1N, Aqueous (1207).
  - a. Save Hydrochloric Acid 0.1N, Aqueous for Step #4.
3. Stain in Alcian Blue Stain 1%, pH 1.0 Aqueous for 30 minutes at room temperature.
4. Rinse sections briefly in Hydrochloric Acid 0.1N, Aqueous.
  - a. **Do not wash in water.** A water wash may change pH level causing nonspecific staining to occur.
5. Blot sections dry with filter paper.
6. Counterstain dried sections with Nuclear Fast Red Stain, Kernechtrot (1255) for 5 minutes.
  - a. Shake solution well before use; do not filter.
7. Rinse well in distilled water.
  - a. See Procedure Note #2.
8. Dehydrate quickly 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:

Sulfated acid epithelial mucin	Blue
Cytoplasm and background	Pink
Nuclei	Pink to red

### PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009.146-147.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 173.
3. Modifications developed by Newcomer Supply Laboratory.