

## Alcian Blue-H&E-Metanil Yellow Stain - Technical Memo

### SOLUTIONS:

	250 ml	500 ml	1 Liter	1 Gallon
Acetic Acid 3%, Aqueous	Part 10017A	Part 10017B		
Alcian Blue Stain 1%, pH 2.5 Aqueous	Part 1003A	Part 1003B		
Hematoxylin Stain, Mayer Modified		Part 1202A	Part 1202B	Part 1202C
Scott Tap Water Substitute		Part 1380A	Part 1380B	Part 1380C
Eosin Y Working Solution		Part 1072A	Part 1072B	Part 1072C
Metanil Yellow Stain, Aqueous	Part 12235B	Part 12235C		

### Additionally Needed:

Alcian Blue pH 2.5, Barrett's Esophagus Control Slides	Part 4023
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Ethyl Denatured, 70%	Part 10844

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

### APPLICATION:

Newcomer Supply Alcian Blue-H&E-Metanil Yellow Stain is a useful diagnostic screening method for Barrett's esophagus and distinguishing from other gastrointestinal conditions/disorders. The use of three separate stains and an eosin counterstain, provides a colorful combination of the gastrointestinal tracts various components.

### METHOD:

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

**Technique:** Paraffin Sections cut at 5 microns

**Solutions:** All solutions are 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:

- 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 #1 and #2.
- Place slides in Acetic Acid 3%, Aqueous for 3 minutes.
- Move slides directly into Alcian Blue Stain 1%, pH 2.5 Aqueous for 15 minutes.
- Wash in running tap water for 5 minutes; rinse in distilled water.
- Stain with Hematoxylin Stain, Mayer Modified for 2 to 4 minutes, depending on preference of nuclear stain intensity.
- Rinse in running tap water until water is clear.
- Blue slides in Scott Tap Water Substitute; 2-3 dips.
- Wash well in running tap water; rinse in distilled water.
- Place in 70% ethyl alcohol for 1 minute.
- Stain with Eosin Y Working Solution for 1 minute.
- Dehydrate in 95% ethyl alcohol for 30 seconds.
- Dehydrate in two changes of 100% ethyl alcohol; 30 seconds each.
- Place in Metanil Yellow Stain, Aqueous for 1 minute.
  - See Procedure Note #3.
- Rinse/dehydrate in two changes of 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Nuclei	Blue
Cytoplasm	Pink-Red
Mucin/Barrett's esophagus goblet cells	Turquoise
Gastric mucin	Some stain faint blue
Collagen	Yellow
Smooth Muscle	Salmon

### PROCEDURE NOTES:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- If over-stained in Metanil Yellow Stain, Aqueous increased background staining will occur.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

- Edgett, Wendi. "Alcian Blue-H&E-Metanil Yellow Stain for Diagnosing Barrett's Esophagus". *Histologic* 37.2 (2004). 35-38
- Staples, Terri. "Alcian Blue-H&E-Saffron Stain for Gastrointestinal Biopsies". *Histologic* 26.1 (1996). 2-3.
- Modifications developed by Newcomer Supply Laboratory.