

## Alcian Blue 1%, pH 2.5 Stain Kit - Technical Memo

### KIT INCLUDES:

	Part 9102A	Part 9102B
Solution A: Acetic Acid 3%, Aqueous	250 ml	500 ml
Solution B: Alcian Blue Stain 1%, pH 2.5 Aqueous	250 ml	500 ml
Solution C: Nuclear Fast Red Stain, Kernechtrot	250 ml	500 ml

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed are two complimentary unstained positive control slides for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

*Individual stain solutions and additional control slides may be available for purchase under separate part numbers at [www.newcomersupply.com](http://www.newcomersupply.com).*

### Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Hyaluronidase	For stromal mucin digestion
Hyaluronidase Buffer	Part 1150 (for stromal mucin digestion)

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

### APPLICATION:

Newcomer Supply Alcian Blue 1%, pH 2.5 Stain Kit procedure, with included hyaluronidase method for stromal mucin digestion, is designed to stain acid epithelial mucin (sialomucin, sulfomucin) as well as stromal (mesenchymal) mucin. The hyaluronidase digestion step is used for further differentiation of acid epithelial from stromal mucin.

### METHOD:

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

**Technique:** Paraffin sections cut at 4 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

### STAINING PROCEDURE:

- If necessary, heat dry tissue sections/slides in oven.
- 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.
- Digestion Step: Proceed to Step #4 if not running Digestion.
  - Two control slides and two patient slides are needed.
  - Label one control slide and one patient slide **"with"**.
  - Label the other control slide and patient slide **"without"**.
  - Prepare Hyaluronidase Digestion Solution and mix well.
 

Hyaluronidase	0.025 gm
Hyaluronidase Buffer (1150)	50 ml
  - Prepare separate Coplin jar of Hyaluronidase Buffer.
  - Preheat both solutions from Steps #2d and #2e to 37°C.
  - Place slides labeled **"with"** in preheated Hyaluronidase Digestion Solution and slides labeled **"without"** in preheated Hyaluronidase Buffer.
  - Incubate both for 2 hours at 37°C.
- Wash all slides in running tap water for 5 minutes; rinse in distilled water. Combine slides for remaining steps.
- Place slides in Solution A: Acetic Acid 3%, Aqueous for 3 minutes.
- Move slides directly into Solution B: Alcian Blue Stain 1%, pH 2.5 Aqueous. Stain for 30 minutes at room temperature or for 15 minutes in a 37°C water bath.

- Wash in running tap water for 10 minutes; rinse in distilled water.
  - See Procedure Note #3.
- Counterstain in Solution C: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
  - Shake solution well before use; do not filter.
- Rinse well in distilled water.
  - See Procedure Note #4
- Dehydrate quickly through two changes of 95% ethyl alcohol and two changes of 100% ethyl alcohol. Clear in three xylene changes, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Acid epithelial mucin	Blue
Stromal (mesenchymal) mucin	Blue
Stromal mucin digestion	Marked loss of staining
Nuclei	Pink-red
Cytoplasm	Pale pink

### PROCEDURE NOTES:

- Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- A brief dip in Solution A: Acetic Acid 3%, Aqueous from Step #5 can be added before water rinses to remove excess Alcian Blue Solution if needed.
- Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
- Sigma Hyaluronidase from Bovine Testes (H3506) is the Hyaluronidase used in the digestion step.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

- Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 145-148.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 172-175.
- Modifications developed by Newcomer Supply Laboratory.