

Alcian Blue Stain 1%, pH 2.5 - Technical Memo

SOLUTION:	250 ml	500 ml
Alcian Blue Stain 1%, pH 2.5 Aqueous	Part 1003A	Part 1003B

Additionally Needed:

Alcian Blue pH 2.5, Umbilical Cord Control Slides	Part 4020	or	Alcian Blue pH 2.5, Multi-Tissue Control Slides	Part 4021
Acetic Acid 3%, Aqueous	Part 10017			
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 product labels.

APPLICATION:

Newcomer Supply Alcian Blue Stain 1%, pH 2.5 Aqueous is designed to stain acid epithelial mucin (sialomucin, sulfomucin) as well as stromal (mesenchymal) mucin in the Alcian Blue Stain 1%, pH 2.5 procedure.

METHOD:

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

Technique: Paraffin sections 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:

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 Notes #1 and #2.
2. Place slides in Acetic Acid 3%, Aqueous (10017) for 3 minutes.
3. Put slides directly into Alcian Blue Stain 1%, pH 2.5 Aqueous and stain for 30 minutes at room temperature or for 15 minutes in a 37°C water bath.
4. Wash in running tap water for 10 minutes; rinse in distilled water.
 - a. See Procedure Note #3.
5. Counterstain in Nuclear Fast Red Stain, Kernechtrot (1255) for 5 minutes.
 - a. Shake solution well before use; do not filter.
6. Rinse well in distilled water.
 - a. See Procedure Note #4
7. 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
Nuclei	Pink-red
Cytoplasm	Pale pink

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. A brief dip in Acetic Acid 3%, Aqueous from Step #2 can be added before rinsing to remove excess Alcian Blue Stain 1%, pH 2.5 Aqueous if needed.
4. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida. *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 118-120.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 172-173.
3. Modifications developed by Newcomer Supply Laboratory.

Alcian Blue/PAS Stain – Technical Memo

SOLUTION:	250 ml	500 ml
Alcian Blue Stain 1%, pH 2.5 Aqueous	Part 1003A	Part 1003B

Additionally Needed:

Alcian Blue/PAS Control Slides	Part 4022
Acetic Acid 3%, Aqueous	Part 10017
Periodic Acid 0.5%, Aqueous	Part 13308
Schiff Reagent, McManus	Part 1371
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 product labels.

APPLICATION:

Newcomer Supply Alcian Blue Stain 1%, pH 2.5 Aqueous, a crucial element in the Alcian Blue/PAS Stain procedure is used to differentiate between acidic epithelial mucins (sialomucin, sulfomucin) and neutral epithelial mucin and is a means of detecting the overall presence of mucins. Acidic mucins are stained with the Alcian Blue technique while neutral mucins and glycogen are stained by the PAS reaction.

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:

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 Notes #1 and #2.
2. Place slides in Acetic Acid 3%, Aqueous (10017) for 3 minutes.
3. Place slides directly into Alcian Blue Stain 1%, pH 2.5 Aqueous for 30 minutes.
4. Wash slides in gently running tap water for 10 minutes; rinse in distilled water.
5. Place slides in Periodic Acid 0.5%, Aqueous (13308) for 10 minutes.
6. Wash slides in running tap water for 5 minutes; rinse in distilled water.
7. Place slides in Schiff Reagent, McManus (1371) for 20 minutes.
8. Wash in lukewarm tap water for 5-10 minutes.
9. Dehydrate 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:

Acid epithelial mucin	Violet
Neutral epithelial mucin	Magenta
Glycogen	Magenta
Stromal (mesenchymal) mucin	Blue

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. Newcomer Supply Schiff Reagent, McManus is stored at room temperature. There is no benefit to store this product at 4°C.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 173-174.
2. Carson, Freida, *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 91-102, 121-123.
3. Modifications developed by Newcomer Supply Laboratory.