

Sulfated Alcian Blue (SAB) Stain – Technical Memo

SOLUTIONS:

	500 ml	1 Liter	1 Gallon
Alcian Blue Stain, Alcoholic	Part 1004A		
Sodium Sulfate 1%, Aqueous	Part 1388A		
Acetic Acid, Glacial, ACS	Part 10010A		
Picric Acid, Saturated Alcoholic	Part 1337A	Part 1337B	Part 1337C
Borax (Sodium Borate) 5%, Saturated Alcoholic	Part 1019A	Part 1019B	
Hematoxylin Stain Set, Weigert Iron	Part 1409B	Part 1409A	
Van Gieson Stain	Part 1404A	Part 1404B	

Additionally Needed:

Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Ethyl Denatured, 80%	Part 10843
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841

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

APPLICATION:

Newcomer Supply Sulfated Alcian Blue (SAB) Stain assists in identifying the extraneous protein deposits of amyloidosis, specifically in myocardial and renal biopsy specimens.

METHOD:

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

Technique: Paraffin sections cut at 8-10 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:

- Prepare the following three fresh solutions:
 - SAB Staining Solution**

Alcian Blue Stain, Alcoholic (1004)	22 ml
Sodium Sulfate 1%, Aqueous (1388)	22 ml
Acetic Acid, Glacial, ACS (10010)	5 ml

Allow to stand for 30 minutes before use.
 - Acetic Acid/Alcohol Solution**

Alcohol, Ethyl Denatured, 95%	44 ml
Distilled Water	44 ml
Acetic Acid, Glacial, ACS	10 ml
 - Picric Acid 2%, Alcoholic Working Solution**

Alcohol, Ethyl Denatured, 80%	30 ml
Picric Acid, Saturated Alcoholic (1337)	10 ml
- 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 in Acetic Acid/Alcohol Solution (Step #1b) for 2 minutes.
- Place slides in SAB Staining Solution (Step #1a) for 2 hours.
- Transfer directly to fresh Acetic Acid/Alcohol Solution (Step #1b) for 2 minutes.
- Wash well in tap water; rinse in distilled water.
- Alkalinize slides in Borax (Sodium Borate) 5%, Saturated Alcoholic (1019) for 30 minutes.
- Wash well in tap water; rinse in distilled water.
- Prepare Working Hematoxylin Stain, Weigert Iron (1409); combine and mix well.
 - Solution A: Ferric Chloride, Aqueous 20 ml
 - Solution B: Hematoxylin 1%, Alcoholic 20 ml
- Stain in Working Hematoxylin Stain, Weigert Iron for 5 minutes.

- Wash in tap water for 10 minutes; rinse in distilled water.
- Differentiate each slide individually; dip in Picric Acid 2%, Alcoholic Working Solution (Step #1c) for 20 seconds.
- Rinse briefly in tap water; 2-3 dips.
- Counterstain with Van Gieson Stain (1404) for 3 minutes.
- 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:

Amyloid deposits	Shades of green; pale to brilliant jade
Fibrin, muscle, cytoplasm	Yellow
Collagen, stroma	Red
Nuclei	Black

Note: Green staining tissues other than amyloid are distinguishable on morphological grounds. Tissue mast cells stain a dense bright green, showing typical cytoplasmic granularity. Red blood cells occasionally appear very pale green; calcium deposits stain a dirty blue/green.

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 using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Lendrum, A. C., W. Slidders, and D. S. Fraser. "Renal Hyalin: A Study of Amyloidosis and Diabetic Fibrinous Vasculosis with New Staining Methods." *Journal of Clinical Pathology* 25 (1972): 373-96.
- Pomerance, Ariela, Gerard Slavin, and Josephine McWatt. "Experience with the Sodium Sulphate-Alcian Blue Stain for Amyloid in Cardiac Pathology." *Journal of Clinical Pathology*, 29 (1976): 22-26.
- Modifications developed by Newcomer Supply Laboratory.