Sulfated Alcian Blue (SAB) Stain – Technical Memo

**SOLUTIONS:**

<table>
<thead>
<tr>
<th>Stain</th>
<th>Volume</th>
<th>1 Liter</th>
<th>1 Gallon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcian Blue Stain, Alcoholic</td>
<td>Part 1004A</td>
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<tr>
<td>Sodium Sulfate 1%, Aqueous</td>
<td>Part 1388A</td>
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<tr>
<td>Acetic Acid, Glacial, ACS</td>
<td>Part 10010A</td>
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<tr>
<td>Picric Acid, Saturated Alcoholic</td>
<td>Part 1337A</td>
<td>Part 1337B</td>
<td>Part 1337C</td>
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<tr>
<td>Borax (Sodium Borate) 5%, Saturated Alcoholic</td>
<td>Part 1019A</td>
<td>Part 1019B</td>
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<tr>
<td>Hematoxylin Stain Set, Weigert Iron</td>
<td>Part 1409B</td>
<td>Part 1409A</td>
<td></td>
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<tr>
<td>Van Gieson Stain</td>
<td>Part 1404A</td>
<td></td>
<td>Part 1404B</td>
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</tbody>
</table>

**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:**

1. 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

2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohol, 10 dips each. Wash with distilled water.
   - a. See Procedure Notes #1 and #2.

3. Place in Acetic Acid/Alcohol Solution (Step #1b) for 2 minutes.
4. Place slides in SAB Staining Solution (Step #1a) for 2 hours.
5. Transfer directly to fresh Acetic Acid/Alcohol Solution (Step #1b) for 2 minutes.
6. Wash well in tap water; rinse in distilled water.
7. Alkalize slides in Borax (Sodium Borate) 5%, Saturated Alcoholic (1019) for 30 minutes.
8. Wash well in tap water; rinse in distilled water.
9. Prepare Working Hematoxylin Stain, Weigert Iron (1409); combine and mix well.
   - a. Solution A: Ferric Chloride, Aqueous 20 ml
   - b. Solution B: Hematoxylin 1%, Alcoholic 20 ml
11. Wash in tap water for 10 minutes; rinse in distilled water.
12. Differentiate each slide individually: dip in Picric Acid 2%, Alcoholic Working Solution (Step #1c) for 20 seconds.
13. Rinse briefly in tap water; 2-3 dips.
14. Counterstain with Van Gieson Stain (1404) for 3 minutes.
15. 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
- Fibrin, muscle, cytoplasm
- Collagen, stroma
- Nuclei

Shades of green; pale to brilliant jade
- Yellow
- Red
- 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:**

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

**REFERENCES:**

3. Modifications developed by Newcomer Supply Laboratory.