
HYALURONIDASE DIGESTION - ALCIAN BLUE

PURPOSE: The digestion procedure is used to increase specificity for various acid mucosubstances in the Alcian blue stain.

CONTROL: Small intestine, appendix, or colon, and a identical slide stained simultaneously without digestion.

FIXATIVE: 10% NBF, Bouin's, or Hollande's.

TECHNIQUE: 4m paraffin sections.

EQUIPMENT: Rinse glassware in DI water. Coplin jars.

REAGENTS:

0.1M Potassium Phosphate, Monobasic:

Potassium phosphate mono	1.36 gm
Distilled water	100.0 ml

Mix well, label with initial and date, store in refrigerator. Stable for 6 months, discard if precipitate forms.

0.1M Sodium Phosphate, Dibasic:

Sodium phosphate di	1.42 gm
Distilled water	100.0 ml

Mix well, label with initial and date, store in refrigerator. Stable for 6 months, discard if precipitate forms.

Buffer Solution:

Potassium phosphate mono,0.1M	94.0 ml
Sodium phosphate di, 0.1M	6.0 ml

Mix well, label with initial and date. Store in refrigerator, stable for 6 months, discard if precipitate forms.

Hyaluronidase Digestion Solution:

Testicular hyaluronidase (Sigma)	0.025gm
Buffer solution	50.0 ml

Mix just before use, discard

Alcian Blue Stain:

See Alcian Blue procedure

SAFETY: Wear gloves, goggles and lab coat. Keep hot, uncapped solutions under the hood. Avoid contact and inhalation.

CARBOHYDRATES

HYALURONIDASE/ALCIAN BLUE

Page: 2 of 2

PROCEDURE:

1. Cut two sets of slides from the same block, label one with hyaluronidase and one without.
2. Deparaffinize and hydrate slides to water.
3. Place one set of slides in Buffer Solution, the other set of slides in Hyaluronidase Solution at 37°C for 1 hour.
4. Wash both sets of slides in running water for 5 minutes.
5. Stain both sets of slides simultaneously with Alcian blue, pH 2.5 following Alcian Blue protocol.

RESULTS:

Extracted areas; hyaluronic acid, chondroitin sulfate A and C: unstained

Unextracted areas blue

Nuclei reddish pink

REFERENCES:

Luna L, Manual of Histologic Staining Methods of the A.F.I.P., 3rd Ed, 1968, pp 171-172, McGraw-Hill, NY.

Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp 174, Battelle Press, Ohio

Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp 212, Churchill Livingstone, NY.

Prepared: _____ By: _____

Approved: _____ By: _____

Downloaded from WebPath: Internet Pathology Laboratory
<http://www-medlib.med.utah.edu/WebPath/webpath.html>

PROCEDURE CARD

HYALURONIDASE DIGESTION - ALCIAN BLUE

CONTROL: Small intestine, appendix, or colon, and an identical slide stained simultaneously without digestion.

PROCEDURE:

1. Cut two sets of slides from the same block, label one with hyaluronidase and one without.
2. Deparaffinize and hydrate slides to water.
3. Place one set of slides in Buffer Solution, the other set of slides in Hyaluronidase Solution at 37°C for 1 hour.
4. Wash both sets of slides in running water for 5 minutes.
5. Stain both sets of slides simultaneously with Alcian blue, pH 2.5 following Alcian Blue protocol; A:1.

RESULTS:

Extracted areas; hyaluronic acid, chondroitin sulphate A and C:	unstained
Unextracted areas	blue
Nuclei	reddish pink

0.1M Potassium Phosphate, Monobasic:

Potassium phosphate, mono	1.36 gm
Distilled water	100.0 ml

Mix well, label with initial and date, store in refrigerator. Stable for 6 months, discard if precipitate forms.

0.1M Sodium Phosphate, Dibasic:

Sodium phosphate di	1.42 gm
Distilled water	100.0 ml

Mix well, label with initial and date, store in refrigerator. Stable for 6 months, discard if precipitate forms.

Buffer Solution:

Potassium phosphate mono, 0.1M	94.0 ml
Sodium phosphate di, 0.1M	6.0 ml

Mix well, label with initial and date. Store in refrigerator, stable for 6 months, discard if precipitate forms.

Hyaluronidase Digestion Solution:

Testicular hyaluronidase	0.025 gm
Buffer solution	50.0 ml

Mix just before use, discard

Alcian Blue Stain:

See Alcian Blue

**0.1M SODIUM PHOSPHATE,
DIBASIC:**

Sodium phosphate di 1.42 gm
Distilled water 100.0 ml

Mix well, label with initial and date,
store in refrigerator. Stable for 6
months, discard if precipitate
forms.

CAUTION: Avoid contact and inhalation.

TECH: _____

DATE: _____

EXPIRATION: _____

BUFFER SOLUTION:

Potassium phosphate mono, 0.1M 94.0 ml
Sodium phosphate di, 0.1M 6.0 ml

Mix well, label with initial and date.
Store in refrigerator, stable for 6
months, discard if precipitate forms.

CAUTION: Avoid contact and inhalation.

TECH: _____

DATE: _____

EXPIRATION: _____

**0.1M POTASSIUM PHOSPHATE,
MONOBASIC :**

Potassium phosphate,
mono 1.36 gm
Distilled water 100.0 ml

Mix well, label with initial and date,
store in refrigerator. Stable for 6
months. Discard if precipitate
forms.

CAUTION: Avoid contact and inhalation.

TECH: _____

DATE: _____

EXPIRATION: _____