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:

forms.

ILL TOLIN I S.			
0.1M Potassium	Phosphate,	Buffer Solution:	
Monobasic:		Potassium phosphate	
Potassium phosphate		mono,0.1M 94.0 ml	
mono	1.36 gm	Sodium phosphate di, 0.1M 6.0 ml	
Distilled water	100.0 ml	Mix well, label with initial and	
Mix well, label with date, store in refrige for 6 months, discard	rator. Stable	date. Store in refrigerator, stable for 6 months, discard if precipitate forms.	

O.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.

Hyaluronidase Digestion Solution:

Date:

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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

PROCEDURE:

1. Cut two sets of slides from the same block, label one with hyaluronidase and one without.

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- 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, chodroitin sulfate A and C: unstained 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:
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PROCEDURE CARD

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RESULTS:

Extracted areas; hyaluronic acid, chodroitin sulphate A and C: unstained Unextracted areas blue

Nuclei reddish pink

O.1M Potassium Phosphate, Monobasic:

Potassium phosphate, mono 1.36 gm Distilled water 100.0 ml

Mix well, label with inital 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 inital 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 pricipate forms.

Hyaluronidase Digestion Solution:

Testicular hyaluronidase 0.025gm Buffer solution 50.0 ml

Mix just before use, discard

Alcian Blue Stain:

See Alcian Blue

O.1M DIBASIO		PHOSPHATE,	O.1M POTASSIUM MONOBASIC:	PHOSPHATE,	
Sodium p	hosphate di	1.42 gm 100.0 ml		e, 1.36 gm	
Mix well, label with inital and date,			Distilled water	•	
store in	refrigerator	Stable for 6 if precipitate	Mix well, label with store in refrigerator months. Discard forms.	r. Stable for 6	
CAUTION: Avoid contact and inhalation.				ot and inhalation	
TECH:			CAUTION: Avoid conta	ct and innaration	
DATE			TECH:		
DATE:			DATE:		
EXPIRATION:			EXPIRATION:		
BUFFER	SOLUTION:				
Potassiur	n phosphate	mono, 0.1M 94.0 ml			
Sodium p	hosphate di,	0.1M 6.0 ml			
Store in	refrigerator	nitial and date. r, stable for 6 recipate forms.			
CAUTION:	Avoid contact	ct and inhalation.			
TECH:					
DATE:					
EXPIRATI	ON:				