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.
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, chodroitin sulfate A and C: unstained
Unextracted areas blue
Nuclei reddish pink

REFERENCES:
PROCEDURE CARD

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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.
**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:________________________

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**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.

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