

ALCIAN BLUE pH 2.5 / PAS

Purpose To differentiate between neutral and acidic mucosubstances.

Principle Acidic mucosubstances are stained by the Alcian blue technique and neutral mucosubstances are stained by the PAS reaction.

Fixative 10% neutral buffered formalin.

Reagents

0.5% Periodic Acid Solution

Periodic acid ... 2.5 g

Distilled water ... 500.0 ml

Schiff's Reagent (purchased)

3% Acetic Acid Solution

Glacial acetic acid ... 3.0 ml

Distilled water ... 97.0 ml

Alcian Blue Solution 1%, pH 2.5

Alcian blue ... 1.0 g

Glacial acetic acid (3%) ... 100.0 ml

Adjust the pH to 2.5. Filter and add a few granules of thymol as a preservative.

Quality Control

Use a control slide of kidney or umbilical cord.

Procedure

1. Deparaffinize and hydrate to water.
2. Mordant in 3% acetic acid solution for 3 minutes.
3. Rinse 5 minutes in running water.
4. Stain in Alcian blue pH 2.5 solution for 30 minutes. This solution can be saved.
5. Wash in running water for 5 minutes.
6. Place in a 0.5% periodic acid solution for 10 minutes.
7. Wash in running water for 5 minutes.
8. Place in Schiff's reagent for 10 minutes.
9. Wash in running water for 5 minutes.
10. Counterstain in hematoxylin for 1-2 minutes.
11. Rinse 5 minutes in running water.
12. Blue, then rinse again in running water.
13. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene, 2 changes each.
14. Mount in synthetic resin.

Results

Exclusively acid mucopolysaccharides ... blue

Neutral polysaccharides ... magenta

Nuclei ... pink

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References

Histotechnology: A Self-Instructional Text, Freida L. Carson, 1990, pp. 130-131.