

Technical Data Sheet

Schiff's Reagent

#26052

1. Mcmanus Pas Method For Glycogen

Fixation:

10% Buffered Neutral Formalin

Section:

Paraffin, @ 6 microns.

Staining Procedure:

Digest using a diastase, hyaluronidase, or sialidase procedure

1. Deparaffinize and hydrate to distilled water
2. Oxidize in Periodic Acid, 0.5%, 5 minutes and rinse in distilled water
3. Strain in Coleman's Feulgen, or **Schiff's Reagent**, 15 minutes and wash in running water to develop the pink color, 10 minutes.
4. Counter stain in Haris Hematoxylin, 6 minutes, or Light Green working Solution, 10 seconds. Light Green is better used when delineation of fungi is required. Prepare the working solution by adding Light Green, 0.2% solution 1:5 with distilled water, proceed to step #7, dehydration. Tap water and ammonia decolorize Light Green.
5. Wash in running water and transfer to Acid Alcohol, 1%, for 3-10 quick dips. Wash again in distilled water.
6. Dip in dilute Ammonia Water, 0.3%, to blue the sections and again wash in running water for ten minutes.
7. Dehydrate in 95% alcohol and 100% alcohol, clear in Xylene, two changes each.
8. Mount.

Results:

Nuclei blue

Fungi red

Background when Light Green is used as the counter stain green, pale

References:

McManus, JEA: Stain Tech, 23 99 (1948)

AFIP Manual of Histologic Staining Techniques; I. G. Luna 3 rd ed. Ed. New York McGaw-Hill Publications. C 1968, p. 160.

Mowry, RW. Annals of the New York Academy of Science: 106: 402 (1963)

2. Pas-Alcian Blue Method For Mucosubstances

Fixation:

10% Buffered Neutral Formalin

Section:

Paraffin, @ 6 microns

Staining Procedure:

1. Deparaffinize and hydrate to distilled water.
2. Stain in Alcian Blue Solution, pH 1.0 or Alcian Blue, pH 2.5 for 30minutes.

3. If Alcian Blue solution, pH 1.0 is used, blot section dry with filter paper. If Alcian Blue solution pH 2.5 is used wash, in water for 5 minutes.
4. Oxidize in Periodic Acid, 1% for 30 minutes. Wash in running water for 5 minutes.
5. Schiff's Reagent, for 10 minutes.
6. Rinse in Sodium Metabisulfite, 0.5% aqueous, three changes, two minutes each. Wash in running water for ten minutes.
7. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, two changes each.
8. Mount with Permount (#17986-01).

Results :

PAS -Alcian Blue pH 2.5 - All polysaccharides and mucosubstances containing hexoses or deoxyhexoses with vicinal glycol groups stain magenta to red.

Mucosubstances staining red include neutral mucosubstances.

Hyaluronic acid, sialomucins and all but the most strongly acidic sulfated mucosubstances stain blue.

PAS -Alcian Blue pH 1.0 - PAS positive same as above. Alciphilic substances at this pH include only the sulphated mucocaccharides.

References:

AFIP Manual of Histological Staining Methods, 3 rd ed. Ed. L. Luna: New York :

McGraw Hill Publications c. 1968, p. 168.

Lev. R. & Spicer S.S. J. Histochem. Cytochem., 12:309. 1964.

3. Periodic Acid Leucofuchsin Method

Fixation:

Zenker Fluid or 10% Formalin

Sections:

Paraffin @ 6 microns.

Staining :

1. Deparaffinized and hydrate to distilled water.
2. Rinse in tap water. Oxidize in Periodic Acid 1% Aqueous for 10 minutes. Wash five minutes in running water.
3. Stain 10 minutes with Schiff's Reagent
4. Pass directly to three successive baths of two minutes each in Sodium Bisulfite 0.05M. Wash in running tap water for 10 minutes.
5. Stain in Weigert's Hematoxylin (*) or Mayer's Acid Hemalum for 2 - 5 minutes, wash in tap water.

*To prepare Weigert's Hamatoxylin: Mix equal parts of Weigert's A and Weigert's B - just before use.

1. Dehydrate in two changes each of 95% and absolute alcohol. Clear through Alcohol-Xylene 1:1 and two changes of Xylene.
2. Mount in Clarita.

Results:

Nuclei Black on Blue

Collagen Pink (Orange if Picric Acid used as counter stain)

Reticulum Purplish Red (Orange-red if Picric Acid is used)

Glycogen- Dark purple red

Epithelial mucin Red -purple to violet

Filbrin- Pink (Pink to violet if the Weigert Fibrin variant was used as a counterstain)

Cytoplasm- Gray, yellow or orange (depending on the counterstain)

References :

Clark, G: Staining Procedures, Williams & Wilkins Co., Baltimore, 3 rd Ed.,c. 1973, p. 156.

4. Microwave Pas-Alcian Blue Method For Mucosubstance

Fixation :

10% buffered Neutral Formalin

Section :

Paraffin, @ 3-5 microns.

Staining Procedure:

1. Deparaffinized and hydrate in the usual manner.
2. Place slides in a loosely covered plastic Coplin jar containing 60 ml Alcian Blue Solution
3. Microwave on medium level 60 seconds. Tightly cover, agitate, and let stand 5 minutes.
4. Wash in distilled water.
5. Place slides in a loosely covered plastic Coplin jar containing 60 ml Periodic Acid, 1% Aqueous.
6. Microwave on medium level 60 seconds, tighten cover, agitate, and let stand one minute.
7. Wash in distilled water.
8. Place slides in a loosely covered plastic Coplin jar containing 60 ml Schiff's Reagent
9. Microwave on medium level 60 seconds, tighten cover, agitate, let stand 30 seconds.
10. Wash in warm running water for three minutes.
11. Place slides in a loosely covered Coplin jar containing 60 ml Gill's Hematoxylin #2
12. Microwave on medium level for 30 seconds.
13. Immediately rinse slides in distilled water.
14. Dip once in Ammonia Water, 1% to blue hematoxylin
15. Wash slides in distilled water, dehydrate in 70%, 95% and two changes of absolute alcohol.
16. Clear in two changes Xylene and mount with Permount

Result:

Acid Mucins turquoise

Neutral Mucins magenta

Combined acid and neutral mucins purple

Nuclei blue

References :

Mathews Karen and James K. Kelly. Dept. of Histology, Foothills Hospital . Calgary , Alberta . Canada . T2N 2T9. Journal of Histotechnology Vol. 12. No 4 Dec. 1989 .