



## 2-3. Modified Heidenhain's Azan (Ref 2)

### Staining Procedures For Plastic Embedded Tissue

Verified at the Applications Laboratory of the Biomedical Division, Sorvall Microtomes

#### SOLUTIONS:

##### Solution A

1.0g Azocarmine B 0.25  
100.0ml Distilled water, cold

*Dissolve, then add:*

1.0ml Glacial acetic acid

*Filter  
or*

1.0g Azocarmine G  
100.0ml Distilled water

Boil, cool, then add:

1.0ml Glacial acetic acid

Filter

##### Solution B

1.0g Aniline blue, W.S  
0.5g Orange G  
8.0ml Glacial acetic acid  
100.0ml Distilled water

Filter

##### Aniline Alcohol

0.1ml Aniline  
100.0ml Ethyl alcohol, 95%

## Acid Alcohol

1.0ml	Acetic acid
100.0ml	Ethyl alcohol, 95%
5%	Phosphotungstic Acid
5.0g	Phosphotungstic acid
100.0ml	Distilled water

## STAINING PROCEDURE:

1. Stain in Solution A at 55°C for 30 minutes. Then cool to 37°C and stain for 2 hours.
2. Wash in distilled water.
3. Rinse in aniline alcohol to remove plastic stain; few dips.
4. Rinse in acid alcohol; few dips.
5. Treat in phosphotungstic acid for 30 minutes to 3 hours.
6. Rinse in distilled water.
7. Stain in Solution B at 60°C for 5-15 minutes.
8. Rinse in distilled water.
9. Dip in 95% ethyl alcohol for 2-3 dips.

Blow dry

Mount.

NOTE: If sections stain too long in Solution B, they begin to lose the orange and overstain with blue.

## RESULTS:

Nuclei	red
erythrocytes	red
muscle	orange
glia fibrils	reddish
mucin	blue
reticulum	dark blue
glomerula stroma	dark blue
collagen	dark blue

Warning: Some of the chemicals used for the staining procedures given in this section may be hazardous if misused. For this reason, read and observe all warnings and cautions provided by the manufacturer for each chemical before proceeding with a staining procedure.

Note: In order to prevent sections from loosening from the slides during staining, all sections should be heat-fixed (60°C to 100°C) to the slides for a minimum of 2-5 minutes prior to staining, preferably at the time the sections are mounted on the slides.

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