**Azan Rapid Stain**

**Description:** This Azan rapid stain is modified from the original Koneff trichrome (1938) staining technique for insect/invertebrate nervous tissue. The rapid protocol neglects the temperature incubation periods. This stain is useful for staining small sections of marine or insect invertebrate tissue, with the ability to differentiate neurons and collagen clearly.

**Fixation:** 10% neutral-buffered formalin, or formol acetic ethanol fixative

**Sectioning:** 6µm paraffin sections

**Solutions:**

Azan stain

 Distilled water ------------------- 300mL

Dissolve each of below before adding next element:

 Phosphotungstic acid -------------1.5g

 Orange G, C.I. 16230 ----------- 3g

 Aniline blue ---------------------- 1.5g

 Acid fuchsin -----------------------4.5g

Add distilled water to make up to 350mL solution.

Keeps for several months to a year. Check for sediment prior to each use.

**Method**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 90% Alcohol – 2 mins
7. Wash in 70% Alcohol – 2 mins
8. Wash in Running Water – 2 mins
9. Stain with Azan stain made up fresh – 5 min
10. Rinse in distilled water rapidly –**3-5 sec**
11. Wash rapidly in 70% ethanol –5 dips
12. Wash rapidly in 100% ethanol –5 dips
13. Wash rapidly in 100% ethanol –5 dips
14. Wash rapidly in 100% ethanol –5 dips
15. Clear in Xylene – 2 mins
16. Clear in Xylene – 2 mins
17. Clear in Xylene – 2 mins
18. Mount slides with coverslips using DePeX

**Results**

Collagen ------------------------------------------- blue

Nuclei ---------------------------------------------- red

Cartilage / bone / mucous / amyloid ----------- shades of blue

Red blood cells / myelin / elastin --------------- yellow or pale pink