Toluidine Blue Staining of Paraffin Sections Used on Plant Sections – March 2013

Modified Significantly from:
Toluidine Blue Staining Protocol for Mast Cells – uses very acidic Toluidine blue solution
http://www.ihcworld.com/_protocols/special_stains/toluidine_blue.htm

Description: Mast cells are found in the connective tissue and their cytoplasm contains granules (metachromatic) composed of heparin and histamine. Toluidine blue should stain mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Metachromasia, tissue elements staining a different color from the dye solution, is due to the pH, dye concentration and temperature of the basic dye. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements.

Fixation: 10% Formalin or FAA

Sections: Plant paraffin sections at 6-8 um.

Solutions and Reagents:

Toluidine Blue Staining Solution: 0.5% Aqueous

Toluidine blue O (Fisher T- 161) ---------- 1 g
Distilled water ---------- 200 ml
Mix to dissolve.

Procedure:

1. Deparaffinize and hydrate sections to distilled water.

TOLUENE	5 minutes
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100% ethanol	2 minutes
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90% ethanol	2 minutes
70% ethanol	2 minutes
50% ethanol	2 minutes
Water	2 minutes

2. Stain sections in toluidine blue solution for 20 seconds
3. Wash in distilled water, 10 dips
4. Wash in distilled water, 10 dips
5. Wash in distilled water, 10 dips

Dehydrate quickly through 95% and 2 changes of 100% alcohol (10 dips each since stain fades quickly in alcohol).

6. Wash in 95% ethanol, 10 dips
7. Wash in 100% ethanol, 10 dips
8. Wash in 100% ethanol, 10 dips
9. TOLUENE 2 minutes
10. TOLUENE 2 minutes plus coverslipping time
11. Coverslip with resinous mounting medium - DPX.