



Toluidine Blue / Fast Green Stain

Basic Info- Toluidine Blue is a **metachromatic** stain. Its staining properties are dramatically altered depending on the following parameters: pH gradient, temperature, light intensity, and solution concentration. Adjustments in any of these areas will yield very different results.

Example: A Basic pH of 9 will yield an intense stain in the extracellular matrix...bright blue/purple color in the GP and AC. An Acidic pH of 4 will stain the nuclei a dark blue/purple color.

REAGENTS:

0.1M Sodium Acetate Buffer

Sodium Acetate, anhydrous (CAS#127-09-3) ----- 13.6 g
Deionized water ----- 1 L

- Stir well until completely dissolved. **Titrate solution to pH 4 using Glacial Acetic Acid.**
- Store at RT or 4C for longer storage

0.4% Toluidine Blue Solution

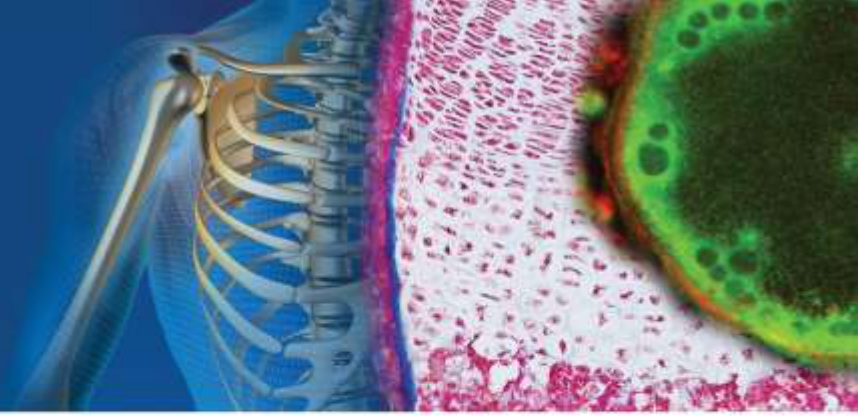
Toluidine Blue O (CAS# 92-31-9) ----- 0.4 g
0.1M Sodium Acetate Buffer ----- 100 ml

- Stir well. Wrap bottle with aluminum foil and store at 4°C.
- Equilibrate the solution to RT before use. Staining is performed at RT, shield from light.

0.02% Fast Green

Fast Green, FCF (CAS# 2353-45-9) ----- 0.05 g
distilled water ----- 250 ml

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PROCEDURE:

1. Deparaffinize slides and rehydrate to deionized water
2. Stain with **0.04% Toluidine Blue Solution** for **10 min**
3. Rinse gently with **3 changes** of deionized water (**30 sec each**)
4. Counterstain with **0.02% Fast Green** solution for **3 min**
5. Rinse gently with **2 changes** of deionized water (**30 sec each**)
6. Dehydrate slides very briefly in **3 changes of 95% EtOH** and **2 changes of 100% EtOH**, **30 seconds each**
7. Clear in **3 changes of Xylene**, **1 minute each**
8. Mount coverslip

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