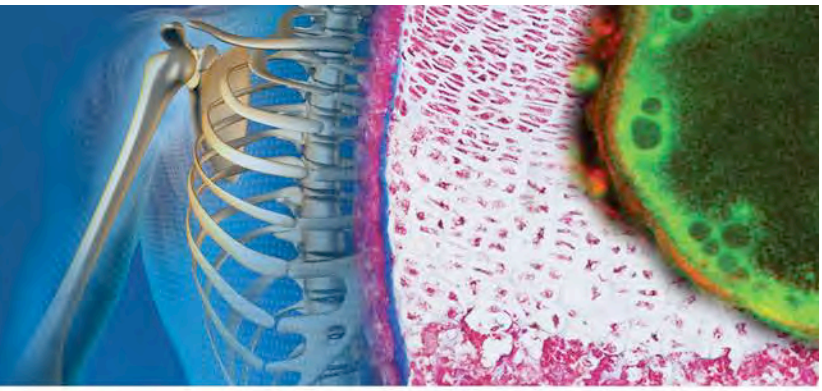


Toluidine Blue / Fast Green Stain

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

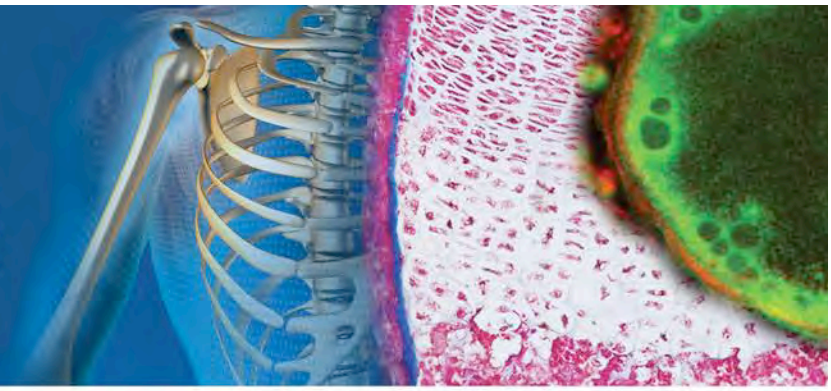
0.4% Toluidine Blue Solution

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 4ºC.
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



Toluidine Blue / Fast Green Stain

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. Dehydate slides very briefly in 3 changes of 95% EtOH and 2 changes of 100% EtOH (30 sec each)
7. Clear in 3 changes of Xylene and coverslip

RESULTS:

Nuclei ---------- dark blue
Cartilage ---------- blue to purple
Background ---------- green