**Toluidine Blue Staining Protocol for Mast Cells**

**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.

**Fixative:** 10% neutral buffered formalin

**Sectioning:** 5µm paraffin wax sections

**Solutions:**

Toluidine Blue Stock Solution:

      Toluidine blue O (Sigma) --------------- 1 g

      70% alcohol ------------------------------ 100 ml

      Mix to dissolve.

Sodium Chloride (1%):

      Sodium chloride --------------------------- 0.5 g

      Distilled water ----------------------------- 50 ml

      Mix to dissolve (make this solution fresh each time). Adjust pH to 2.0~2.5 using glacial acetic acid or HCl.

Toluidine Blue Working Solution (pH 2.0~2.5):

      Toluidine blue stock solution -------------- 5 ml

      1% Sodium chloride, pH 2.3 --------------- 45 ml

      Mix well. The pH should be around 2.3 and less than 2.5

      Make this solution fresh and discard after use. pH higher than 2.5 will make staining less contrast.

**Procedure:**

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 sections in toluidine blue working solution for 2-3 minutes
10. Wash in distilled water – 2 mins
11. Dehydrate quickly in 95% ethanol.
12. Dehydrate in100% alcohol (10 dips each)
13. Dehydrate in100% alcohol (10 dips each)
14. Clear in Xylene – 2 mins
15. Clear in Xylene – 2 mins
16. Clear in Xylene – 2 mins
17. Mount slides with coverslips using DePeX

**Results:**

      Mast cells --------------------------- violet/red purple.

      Background ------------------------- blue