

## Toluidine Blue Staining Kit, 0.5%, Phosphate Buffer Method

**Catalog No.: RA20123**

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### Basic Information

<b>Product name</b>	Toluidine Blue Staining Kit, 0.5%, Phosphate Buffer Method
<b>Sizes</b>	100 mL
<b>Storage</b>	RT, keep away from light
<b>Shipping</b>	RT
<b>Validity</b>	12 months

### Product Introduction

Toluidine Blue O is a commonly used synthetic dye belonging to the quinone-imine dye class. These dyes mainly contain two chromophores—amino groups and quinoid benzene rings—which contribute to color development. The cations in toluidine blue are responsible for staining, as they bind to acidic substances in tissue cells. Toluidine blue also contains two auxochromes that promote ionization and salt formation, enhancing the dye's affinity for tissues and enabling cellular staining. It can stain nuclei blue. Additionally, mast cells contain metachromatic substances such as heparin and histamine, which stain metachromatically purple-red when exposed to toluidine blue.

EnkiLife Toluidine Blue O Stain (0.5%, Phosphate Buffer Method) is commonly used for staining cells and mast cells.

### Materials Required (Not Supplied)

1. Distilled or deionized water, graded ethanol series, glacial acetic acid, acetone.
2. Xylene or eco-friendly dewaxing and clearing agent, neutral balsam.

### Experimental procedure

#### (I) Mast Cell Staining

1. Dewax sections and bring to distilled water.
2. Immerse sections in toluidine blue staining solution for 5–30 min, depending on section thickness and tissue type.
3. Gently rinse with distilled or deionized water for 3–5 min.
4. (Optional) Differentiate with 0.5% glacial acetic acid until nuclei and granules are clearly visible.
5. Dehydrate quickly with 95% and absolute ethanol, clear with xylene or clearing agent, and mount.

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6. Staining Results: Mast cells appear purple-red; background appears light blue.

### (II) Cartilage Staining

1. Immerse paraffin sections in xylene twice, 15 min each.
2. Rinse through graded ethanol series, 1 min each; rinse with tap water for 2 min.
3. Immerse in Toluidine Blue O Stain (0.5%, Phosphate Buffer Method) for 10–15 min.
4. Rinse with tap water for 2 min; blot dry with filter paper.
5. Differentiate with acetone until chondrocytes appear clearly purple-blue; dehydrate through graded ethanol.
6. Clear with xylene or clearing agent; mount with neutral balsam.
7. Staining Results: Cartilage and osteoblasts appear purple-red; background appears light blue.

### (III) Cell Smear Staining

1. Dilute toluidine blue staining solution with 20% ethanol to ~0.1%.
2. After preparing the smear, immediately fix in 95% ethanol for 15 s; place on absorbent paper.
3. Apply 1–2 drops of diluted toluidine blue staining solution, cover with a coverslip to allow dye penetration.
4. After 10 – 15 s, stand the slide upright and apply slight pressure to remove excess dye with absorbent paper. No drying needed—proceed directly to microscopy.
5. Staining Results: Nuclei and lymphocytes appear deep blue; nucleoli appear purple-red; red blood cells appear orange-red; cytoplasm and monocytes appear light blue.

### (IV) In Situ Hybridization Staining

1. Dilute with distilled or deionized water to the desired concentration—typically >1:100 based on experience.
2. Briefly immerse slides in diluted staining solution.
3. Rinse several times in distilled or deionized water.
4. Proceed with coverslip mounting as needed.

## **Notes**

1. For difficult-to-stain tissues such as gastric mucosa or cartilage, extend immersion time in toluidine blue staining solution as needed.
2. For your safety and health, wear a lab coat and disposable gloves during operation.

**This product is for research use only!**