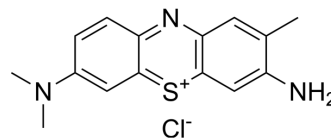


Toluidine Blue

Cat. No.:	HY-D0220
CAS No.:	92-31-9
Molecular Formula:	C ₁₅ H ₁₆ ClN ₃ S
Molecular Weight:	305.83
Target:	Fluorescent Dye
Pathway:	Others
Storage:	4°C, sealed storage, away from moisture and light * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 20.83 mg/mL (68.11 mM; ultrasonic and warming and heat to 80°C)
H₂O : 5 mg/mL (16.35 mM; ultrasonic and warming and heat to 60°C)

Solvent	Mass	Concentration		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	3.2698 mL	16.3490 mL	32.6979 mL
	5 mM	0.6540 mL	3.2698 mL	6.5396 mL
	10 mM	0.3270 mL	1.6349 mL	3.2698 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

Toluidine Blue (Toluidine Blue O) is an alkaline quinonimine dye (vivo dye) with high affinity for acidic tissue components, staining nuclei blue and polysaccharides purple. Toluidine Blue shows heterostaining properties for mast cells, mucins and chondrocytes. Toluidine Blue can stain different components of plant tissues and cells in different colours. Toluidine Blue is also used as a diagnostic aid to identify malignant lesions, such as cancer^{[1][2][3]}.

In Vitro

Guidelines (Following is our recommended protocol. This protocol only provides a guideline, and should be modified according to your specific needs).
Detection of infiltrating Mast cells^[1]:

- Tissue processing
 - 1) Immersed tissue samples in 10% buffered formalin for 24 h.
 - 2) Remove tissues from the fixative and place in 70% alcohol.
 - 3) Embed in paraffin: sequentially incubate in 70, 85, 95, and 100% ethanol, xylene, and molten paraffin (30 min incubation each), and set tissues in paraffin blocks.
 - 4) Cut paraffin-embedded tissue in 5-7 µm sections and place onto glass slides.
 - 5) Bake the slides for a minimum of 1 h at 55°C before use.

2. Tissue staining

- 1) De-wax paraffin-embedded tissue sections by immersing slides 5 min in xylene (or Histo-Clear), and rehydrate tissue by sequential immersions (5 min each) in 100%, 95%, and 70% ethanol and a final 2 min immersion in distilled water.
- 2) Place the slides in Harris' hematoxylin solution for 70 s.
- 3) Briefly wash the excess of hematoxylin by immersing the slides two or three times in clean tap water. Briefly tap the slides on paper towels to remove excess water.
- 4) Immerse slides briefly three times in 70% alcohol, and wash again in clean tap water for 3 min. Remove excess water.
- 5) Place slides in Scott's bluing solution for up to a minute. This step gently transforms hematoxylin into an insoluble blue color within the nucleus.
- 6) Inspect the slides under the bright-light microscope to ensure the nuclei are properly stained.
- 7) Wash slides in clean tap water for 3 min, and remove excess water.
- 8) Immerse slides in clean ~1.1% Toluidine Blue solution for 4 min.
- (Note 1).9) Wash slides in clean tap water for 3 min and remove excess water.
- 10) Quickly dip the slides three times in one batch of 70% ethanol and then dip them five times in 5% eosin solution.
- 11) Dehydrate the slides by successive immersion in 70% (2 min), 95% (twice, 2 min each), and 100% ethanol (twice, 2 min each); conclude this step by immersing the slides in xylene (or Histo-Clear) (twice, 3 min each).
- 12) Permanently mount each slide with a clean coverslip using xylene-based mounting medium.
- 13) Let slides dry for a minimum of 60 min before analyzing them under the bright-light microscope.

Note 1: 1.1% Toluidine Blue solution: mix 1.1 g Toluidine Blue with 100 mL 0.1 M sodium acetate buffer pH 4. Stir well. Adjust the pH to 2.0-2.5 by adding drops of 1 M hydrochloric acid. Staining is performed at 25 °C, protected from light. Ready to use.

Application of Toluidine Blue^{[2][3]}:

1. Connective tissue mucins, especially acid mucins. The tissue stains purple to red, while the background is stained blue.
2. Mast cell granules stain purple in color due to the presence of heparin and histamine.
3. Amyloid stain blue but under polarized light they give a bright red birefringence.
4. Endocrine cell granules are stained purple to red (concentration of stain is 0.01%).
5. Sulfatides stain red brown or yellow. Only lipids that are sufficiently acidic to induce a metachromatic shift are stained.
6. *Corynebacterium diphtheriae* contains granules with polymerized inorganic polyphosphate, which stains red violet color.
7. *Helicobacter* stains dark blue against a variably blue background (concentration of stain is 1%).
8. Toluidine Blue can be used to stain frozen section because of the rapidity of the staining procedure (10-20 s) and better clarity of the cells.
9. Toluidine Blue can also dye the lignins of the plant blue-green, the phloem blue-purple, and the rest of the plant pale blue-green.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

- [1]. Puebla-Osorio N, et al. Detection of Infiltrating Mast Cells Using a Modified Toluidine Blue Staining. *Methods Mol Biol.* 2017;1627:213-222.
- [2]. O'Brien T P, et al. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*, 1964, 59(2): 368-373.
- [3]. Sridharan G, et al. Toluidine blue: A review of its chemistry and clinical utility. *J Oral Maxillofac Pathol.* 2012;16(2):251-255.

Caution: Product has not been fully validated for medical applications. For research use only.

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