

Toluidine blue staining

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Subsequent to immunohistological reaction, counterstaining of tissue sections with the thiazin dye toluidine blue (Toluidine blue O) is an alternative to other dyes (e.g. haematoxylin, carmine, methyl green) for nuclear staining. Moreover, the staining procedure is rapid and easily performed, thus, toluidine blue is helpful and widely employed for any kind of overview stainings. There exist formulations for cryostat and paraffin sections as well as for sections cut from resin embedded tissue.*

Toluidine O (overview staining, paraffin sections)

Chemicals	Chemical solution
Toluidine blue O (C.I. 52040) Ethanol Distilled water	<ul style="list-style-type: none">Toluidine blue dye solution: 0.1 g toluidine blue dissolved in 100.0 mL distilled water
Staining procedure Immuno-stained sections are passed through distilled water and stained: <ul style="list-style-type: none">Toluidine blue dye solution 10-20 mindistilled water several rinses under microscopic control96% ethanol 1 min Slides are dehydrated in absolute ethanol, cleared in xylene or xylene substitute and mounted in resinous medium under coverglass	

Toluidine O (nuclear staining according to Mayer; Metzner)

Chemicals	Chemical solution
Toluidine blue O (C.I. 52040) Iron ammonium sulfate (ammonium ferric sulfate) Ethanol	<ul style="list-style-type: none">Toluidine blue dye solution: 0.1 g toluidine blue dissolved in 100.0 mL distilled waterIron ammonium sulfate solution: 3.0-5.0 g dissolved in distilled water

* Dyes and other chemicals in histological staining can be toxic. They must be handled with care

Distilled water	
Staining procedure	
Immuno-stained sections are passed through distilled water and stained:	
– Iron ammonium sulfate solution	45 min
– distilled water	rinse
– toluidine blue dye solution	10-20 min
– differentiate in 50% ethanol	until dye veils are no longer seen
– 96% ethanol	1 min
Slides are dehydrated in absolute ethanol, cleared in xylene or xylene substitute and mounted in resinous medium under coverglass	

Toluidine O (overview staining, semithin resin sections)

Chemicals	Chemical solution
Toluidine blue O (C.I. 52040) Sodium borate Ethanol Distilled water	<ul style="list-style-type: none"> Toluidine blue dye solution: 1.0 g sodium borate dissolved in 100.0 mL distilled water <i>plus</i> 1.0 g toluidine blue stir until the dye is dissolved filter prior to use
Staining procedure	
Semithin sections are mounted on a glass slide and dried on a slide warmer. Dry sections are stained:	
– Toluidine blue dye solution	1-2 min while the slide is still warmed, then cool down
– distilled water	several rinses
– 96% ethanol	2 dips
– absolute ethanol	2 x 1 min
Slides are passed from absolute ethanol into xylene or xylene substitute and mounted in resinous medium under coverglass	

References for further readings

Metzner R (1907)
 Metzner R (1915)
 Richardson KC *et al.* (1960)
 Trump BF *et al.* (1961)
 Mercer EH (1963)
 Romeis B (1968)

Full citation of publications is given in chapter *References*

link: <https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf>

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10.09.2006