

## Toluidine Blue Stain for Mast Cells - Technical Memo

**SOLUTION:**

Toluidine Blue Stain 0.1%, Aqueous	<b>250 ml</b> Part 14027A	<b>500 ml</b> Part 14027B	<b>1 Gallon</b> Part 14027D
------------------------------------	------------------------------	------------------------------	--------------------------------

Additionally Needed:

Mast Cell Control Slides	Part 4410	or	Mast Cell, Animal Control Slides	Part 4412
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			

**For storage requirements and expiration date refer to individual product labels.**

**APPLICATION:**

Newcomer Supply Toluidine Blue Stain for Mast Cells is for the demonstration of mast cells, characterized as cells filled with basophilic granules, associated with inflammation and allergic reactions, which stain metachromatically with toluidine blue.

**METHOD:**

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)  
**Technique:** Paraffin sections cut at 4 microns  
**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

**STAINING PROCEDURE:**

1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
  - a. See Procedure Notes #1 and #2.
2. Place slides in Toluidine Blue Stain 0.1%, Aqueous for 10 minutes.
3. Rinse well in distilled water.
4. Dehydrate quickly through two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
  - a. See Procedure Note #3.

**RESULTS:**

Mast cells	Deep blue-violet
Background	Blue

**PROCEDURE NOTES:**

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. Metachromasia of mast cell granules is stable and staining will be maintained during dehydration steps.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

## Toluidine Blue Stain for Mohs Technique - Technical Memo

<b>SOLUTION:</b>	<b>250 ml</b>	<b>500 ml</b>	<b>1 Gallon</b>
Toluidine Blue Stain 0.1%, Aqueous	Part 14027A	Part 14027B	Part 14027D

<u>Additionally Needed:</u>	
Alcohol, Ethyl Denatured, 70%	Part 10844
Alcohol, Ethyl Denatured, 95%	Part 10842
Alcohol, Ethyl Denatured, 100%	Part 10841
Xylene, ACS	Part 1445

**For storage requirements and expiration date refer to individual bottle labels.**

**APPLICATION:**

Newcomer Supply Toluidine Blue Stain for Mohs Technique provides a rapid staining method for Mohs micrographic surgery (MMS), useful when evaluating frozen skin samples for basal cell carcinoma (BCC). Toluidine Blue imparts an identifiable staining pattern if BCC is present that will highlight islands of blue staining basal cell carcinoma and metachromatically stain surrounding mucopolysaccharides/stroma pink.

**REFERENCES:**

1. Arnon, Ofer, Ronald Rapini, Adam Mamelak, and Leonard Goldberg. "Mohs Micrographic Surgery: Current Techniques." *IMAJ* 12 (2010): 431-35.
2. Gross, Kenneth G. *Mohs Surgery: Fundamentals and Techniques*. St. Louis: Mosby, 1999. 125-138.
3. Modifications developed by Newcomer Supply Laboratory.

**METHOD:**

**Fixation:** 70% Ethyl Alcohol (Part 10844)  
**Technique:** Frozen sections cut at 5-7 microns on adhesive slides  
**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

**STAINING PROCEDURE:**

1. Fix tissue sections in 70% Ethyl Alcohol for 30-60 seconds.
  - a. See Procedure Note #1.
2. Wash well in distilled water.
3. Stain slides in Toluidine Blue Stain 0.1%, Aqueous for 30-60 seconds, depending on preference of stain intensity.
4. Wash gently in distilled water.
5. Dehydrate quickly through one change of 95% ethyl alcohol; 1 quick dip and then two changes 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
  - a. See Procedure Note #2.

**RESULTS:**

Islands of basal cell carcinoma	Deep blue to purple
Surrounding mucopolysaccharides/stroma	Pink to magenta
Background	Blue
Nuclei	Dark blue

**PROCEDURE NOTES:**

1. Section thickness and fixation timing may affect staining quality.
2. Alcohol will work as a differentiator. Proceed quickly through dehydration steps to maintain Toluidine Blue stain.
3. If using a xylene substitute, closely follow the manufacturer's recommendation for clearing step.