

# Helicobacter, Toluidine Blue/Alcian Yellow Stain Kit- Technical Memo

## KIT INCLUDES:

	<b>Part 9130A</b>
Solution A: Periodic Acid 1%, Aqueous	250 ml
Solution B: Sodium Metabisulfite 5%, Acidified	250 ml
Solution C: Alcian Yellow Stain 1%, Alcoholic	250 ml
Solution D: Toluidine Blue Stock Stain 1%, Aqueous	10 ml
Solution E: Sodium Hydroxide 3%, Aqueous	10 ml

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

*Individual stain solutions and control slides may be available for purchase under separate part numbers at [www.newcomersupply.com](http://www.newcomersupply.com).*

## Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Acetic Acid 3%, Aqueous	Part 10017 (for more intense Alcian Yellow staining)

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

## APPLICATION:

Newcomer Supply Helicobacter, Toluidine Blue/Alcian Yellow Stain Kit procedure is used for screening and detection of *Helicobacter pylori* in gastrointestinal biopsy specimens.

## METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

## 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 Note #1.
2. Oxidize in Solution A: Periodic Acid 1%, Aqueous for 10 minutes.
3. Wash slides well with tap water.
4. Treat slides with Solution B: Sodium Metabisulfite 5%, Acidified for 5 minutes.
5. Wash in tap water for 5 minutes.
  - a. See Procedure Note #2.
6. Stain in Solution C: Alcian Yellow Stain 1%, Alcoholic for 5 minutes.
7. Wash well in tap water.
8. Prepare fresh Toluidine Blue Stain Working Solution; combine and mix well.
  - a. Distilled Water 50 ml
  - b. Solution D: Toluidine Blue Stock Stain 1%, Aqueous 0.5 ml
  - c. Solution E: Sodium Hydroxide 3%, Aqueous 2 drops
9. Stain in fresh Toluidine Blue Stain Working Solution for 3-5 minutes depending on preference of stain intensity.
10. Wash well in tap water.
11. Allow slides to air-dry completely.
12. Clear dried slides in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
  - a. See Procedure Note #3.

## RESULTS:

<i>Helicobacter pylori</i>	Blue
Mucin	Yellow
Background	Pale blue

## PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. For a more intense Alcian Yellow stain, add a step of Acetic Acid 3%, Aqueous (10017) for 1-3 minutes prior to Step #6. Do not rinse slides and proceed directly into Solution C: Alcian Yellow Stain 1%, Alcoholic.
3. The elimination of the dehydration steps is necessary to retain organism staining.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

## REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 225-226.
2. Leung, Jennifer, Kevin Gibbon and Robert Vartaniam. "Rapid Staining Method for *Helicobacter pylori* in Gastric Biopsies." *The Journal of Histotechnology* 19.12 (1996): 131-132.
3. Modifications developed by Newcomer Supply Laboratory.