

AFB, Ziehl-Neelsen Stain Kit - Technical Memo

KIT INCLUDES:

Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen
Solution B: Acid Alcohol 1%
Solution C: Methylene Blue Stain 0.14%, Alcoholic

Part 9101A

250 ml
250 ml
250 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 additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

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 bottle labels.

APPLICATION:

Newcomer Supply AFB, Ziehl-Neelsen Stain Kit procedure demonstrates the presence of acid-fast mycobacteria in tissue sections. Phenol is employed to render bacteria cell walls permeable to the fuchsin stain. The use of weak acid for differentiation removes excess stain from tissues, but will not remove stain from acid-fast organisms.

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.

PRESTAINING PREPARATION:

1. Filter Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen with high quality filter paper when a sheen develops on solution surface.

STAINING PROCEDURE:

2. 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.
3. Stain in Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen for 15 minutes at room temperature. Keep solution covered.
 - a. See Procedure Note #3.
4. Rinse in running tap water for 2 to 3 minutes.
5. Differentiate in Solution B: Acid Alcohol 1% until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
 - a. Check microscopically to ensure differentiation is complete.
6. Wash in running tap water 2 to 3 minutes; rinse in distilled water.
7. Counterstain in Solution C: Methylene Blue Stain 0.14%, Alcoholic; 3 to 6 dips. Rinse in tap water followed by distilled water rinse.
 - a. Check microscopically. Sections should be pale blue.
 - b. See Procedure Notes #4 and #5.
8. Wash in running tap water for 1 minute; rinse in distilled water.
9. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Acid-fast bacilli	Bright red
Background	Pale blue

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. Sections can remain in Carbol Fuchsin Stain, Ziehl-Neelsen for up to 60 minutes. Additional differentiation may be required in Step #5.
4. If sections are over-stained, remove methylene blue with acid alcohol, rinse thoroughly and repeat Step #7 with fewer dips. Over-counterstaining can mask organisms.
5. If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
6. 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. 218.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 236-237.
3. Modifications developed by Newcomer Supply Laboratory.