2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • info@newcomersupply.com

Part 9101
Revised July 2018

AFB, Ziehl-Neelsen Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9101A**
Solution A: Carbol Fuchsin Stain, Ziehl-Neelsen	250 ml
Solution B: Acid Alcohol 1%	250 ml
Solution C: Light Green SF Yellowish Stain 0.1%, Aqueous	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 is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Acid-fastness is a physical property of certain bacteria and cellular structures. Carbol Fuchsin Stain, Ziehl-Neelson, combines phenol and basic fuchsin that works to permeate the lipoid capsule of acid-fast organisms and renders them resistant to acid alcohol decolorization.

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 filter paper whenever a thick 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.
6. Wash in running tap water 3 to 5 minutes; rinse in distilled water.
7. Counterstain in Solution C: Light Green SF Yellowish Stain 0.1%, Aqueous; 2-5 dips.
8. Rinse with one quick dip in distilled water or proceed directly to Step #9 without a distilled water rinse.
9. Dehydrate quickly 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	Green

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 without adverse effect. Additional differentiation may be required in Step #5.
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. 218-220.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
3. Modifications developed by Newcomer Supply Laboratory.
