

AFB, Basic Fuchsin, Ellis & Zabrowarny Stain Kit - Technical Memo

KIT INCLUDES:

	Part 91012A
Solution A: Basic Fuchsin Stain 1%, Alcoholic	250 ml
Solution B: Acid Alcohol 3%	250 ml
Solution C: Methylene Blue Stain 0.25%, 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
Coplin Jar, Plastic	Part 5184 (for microwave modification)

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

APPLICATION:

Newcomer Supply AFB, Basic Fuchsin, Ellis & Zabrowarny Stain Kit procedure, with included microwave modification, provides a safer method for staining acid-fast bacilli without the use of phenol. A surfactant substitution is made for phenol which renders the bacterial cell wall permeable to the stain, with results comparable to the traditional Ziehl-Neelsen method.

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:

- Preheat Solution A: Basic Fuchsin Stain 1%, Alcoholic to 60°C in an oven or water bath. **(Skip if using Microwave Modification.)**

STAINING PROCEDURE:

- 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.
 - See Procedure Notes #1 and #2.
- Place slides in preheated Solution A: Basic Fuchsin Stain 1%, Alcoholic for 15 minutes.

Microwave Modification: See Procedure Note #3.

 - Place slides in a plastic Coplin jar containing Solution A: Basic Fuchsin Stain 1%, Alcoholic and microwave for 1 minute at 70°C.
- Rinse slides well in running tap water for 2 to 3 minutes.
- Differentiate in Solution B: Acid Alcohol 3% until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
- Wash in running tap water for 1 minute.
- Counterstain **one slide at a time** in Solution C: Methylene Blue Stain 0.25%, Aqueous for 15 to 30 seconds.
 - Dip slides a few times in counterstain; rinse in tap water, followed by a distilled water rinse.
 - Check microscopically. Sections should be pale blue.
 - See Procedure Notes #4 & #5.

- Wash in running tap water for 1 minute; rinse in distilled water.
- 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	Red
Nuclei and background	Shades of blue

PROCEDURE NOTES:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
- Organisms may be masked if over-stained. If section is over-stained, remove methylene blue with acid alcohol, rinse thoroughly, and repeat methylene blue step (Step #7) with fewer dips.
- If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
- Solution A: Basic Fuchsin Stain 1%, Alcoholic does not contain phenol and can be readily disposed of.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Ellis, R C, and L A Zabrowarny. "Safer Staining Method for Acid Fast Bacilli." *Journal of Clinical Pathology* 46.6 (1993): 559-560.
- Modifications developed by Newcomer Supply Laboratory.