

Feulgen Reaction - Technical Memo

SOLUTIONS:	125 ml	250 ml	500 ml	1 Liter	4 Liters
Hydrochloric Acid 20%, Aqueous			Part 12087A	Part 12087B	
Schiff Reagent, McManus	Part 1371A	Part 1371E	Part 1371B	Part 1371C	Part 1371D

Additionally Needed:

Normal Tonsil Custom Tissue Slides	Part CT39790A
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Light Green SF Yellowish Stain 0.2%, Aqueous	Part 12202

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

APPLICATION:

The Newcomer Supply Feulgen Reaction procedure is used for the demonstration of DNA (deoxyribonucleic acid) in tissue sections.

METHOD:

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

a. See Procedure Note #1.

Technique: Paraffin sections cut at 4 microns

Solutions: All solutions manufactured by Newcomer Supply, Inc.

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

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Prepare Hydrochloric Acid Working Solution; combine and mix well.
 - a. Hydrochloric Acid, 20% Aqueous 16 ml
 - b. Distilled Water 24 ml
 - c. Preheat and maintain Hydrochloric Acid Working Solution at 60°C.

STAINING PROCEDURE:

3. 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 #2 and #3.
4. Hydrolyze sections in preheated Hydrochloric Acid Working Solution (Step #2) at 60°C for 10 minutes.
 - a. See Procedure Notes #4 and #5.
5. Place slides directly in Schiff Reagent, McManus for 45 minutes.
6. Wash in running tap water for 5 minutes; rinse in distilled water.
7. Counterstain in Light Green SF Yellowish Stain 0.2%, Aqueous (12202) for 1 minute.
8. 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:

DNA	Red-purple
Nuclei	Red-purple
Background	Green

PROCEDURE NOTES:

1. Bouin fixed tissue is unsatisfactory for use with Feulgen reaction.
2. Drain slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during procedure.
4. For optimal results preheat and maintain Hydrochloric Acid Working Solution at 60°C during the hydrolysis process.
5. Prolonged exposure to hydrochloric acid may over-hydrolyze sections with poor staining results.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for the clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008.224-225.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-Instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 126-127.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 150.
4. Modifications developed by Newcomer Supply Laboratory.