

B-5 Fixative Modified, Zinc Chloride - Technical Memo

SOLUTION:

B-5 Fixative Modified, Zinc Chloride

1 Liter
Part 1015A

6 X 1 Liter
Part 1015A

1 Gallon
Part 1015C

Additionally Needed:

Formaldehyde 37-40%, ACS

Part 1089

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

APPLICATION:

Newcomer Supply B-5 Fixative Modified, Zinc Chloride provides an alternative to the classic B-5 fixative with mercuric chloride. Environmental concerns and costly requirements for disposal of mercury-containing fixatives have led to the modification of the original B-5 formulation by substituting zinc chloride for mercuric chloride. This maintains the benefits of B-5 fixation with no concern for mercury pigment.

B-5 Fixative Modified, Zinc Chloride will demonstrate clear nuclear detail, preserve immunohistochemical (IHC) staining and is a good fixative choice for bone marrow, lymph nodes, spleen and other hematopoietic tissues.

METHOD:

Fixation:

Bone Marrow: Recommended fixation time for bone marrow clot is a minimum of 2 hours; for bone marrow biopsy a minimum of 3 hours.

a. See Procedure Note #1.

Lymph Nodes and Small Biopsies: A minimum of 4 hours is recommended. Small nodes (5 mm or less) should be halved. If larger, dissect in such a way that no piece is thicker than 3 mm.

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

FIXATION PROCEDURE:

- B-5 Fixative Modified, Zinc Chloride Working Solution:
 - B-5 Fixative Modified, Zinc Chloride 40 ml
 - Formaldehyde 37-40%, ACS 4 ml
- Combine solutions directly before use. Fix tissue in this fresh 10:1 working solution for a minimum of 2 to 4 hours depending upon type of tissue.
- Hold tissue specimens in B-5 Fixative Modified, Zinc Chloride Working Solution until ready to process or for a maximum of 72 hours.
 - See Procedure Note #2.
- Wash fixed tissue thoroughly in running tap water to remove residual zinc chloride.
- Place on tissue processor in Formalin 10%, Phosphate Buffered (Part 1090) fixation step.

PROCEDURE NOTES:

- Nitric acid solutions are not recommended as a decalcification agent following B-5 Fixative Modified, Zinc Chloride fixation.
- Extended storage of tissue in B-5 Fixative Modified, Zinc Chloride Working Solution is not recommended. After a maximum fixation time of 72 hours, transfer B-5 fixed wet tissue to 70% Ethyl Alcohol (Part 10844) for long-term storage purposes.
- Alternative uses of B-5 Fixative Modified, Zinc Chloride:
 - Stock solution as working solution: Place tissue directly into undiluted B-5 Fixative Modified, Zinc Chloride for 2 to 4 hours. Proceed to Step #3c.
 - Stock solution with Acetic Acid, Glacial, ACS (Part 10010) for working solution: Combine solutions directly before use:

B-5 Fixative Modified, Zinc Chloride	40 ml
Acetic Acid, Glacial, ACS	4 ml

 Place tissue in this fresh 10:1 working solution for 2 to 4 hours.
 - Rinse tissue thoroughly in running tap water. Proceed with Formalin 10%, Phosphate Buffered (Part 1090) fixation on tissue processor.
 - Without the addition of formaldehyde to B-5 Fixative Modified, Zinc Chloride, the quality of tissue fixation may be compromised.
- Neutralize B-5 Fixative Modified, Zinc Chloride, stock or working solutions with magnesium hydroxide/oxide, sodium carbonate or sodium bicarbonate to precipitate zinc at pH 7.0-8.0. Separate solids from liquid and dispose of according to local and state environmental regulations.
 - Approximately 100 grams of sodium bicarbonate will neutralize/precipitate zinc from 1 liter of B-5 Fixative Modified, Zinc Chloride.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 69.
- Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 17-19.
- Dapson, Janet Crookham, and Richard Dapson. *Hazardous Materials in the Histopathology Laboratory: Regulations, Risks, Handling, and Disposal*. 4th ed. Battle Creek, MI: Anatech, 2005. 148-149, 279.
- Modifications developed by Newcomer Supply Laboratory.