B-5 Fixative Modified, Zinc Chloride - Technical Memo

SOLUTION:

<table>
<thead>
<tr>
<th>B-5 Fixative Modified, Zinc Chloride</th>
<th>1 Liter</th>
<th>6 X 1 Liter</th>
<th>1 Gallon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1015A</td>
<td></td>
<td>Part 1015A</td>
<td>Part 1015C</td>
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</tbody>
</table>

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:

1. B-5 Fixative Modified, Zinc Chloride Working Solution:
   a. B-5 Fixative Modified, Zinc Chloride 40 ml
   b. Formaldehyde 37-40%, ACS 4 ml
2. 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.
3. Hold tissue specimens in B-5 Fixative Modified, Zinc Chloride Working Solution until ready to process or for a maximum of 72 hours.
   a. See Procedure Note #2.
4. Wash fixed tissue thoroughly in running tap water to remove residual zinc chloride.
5. Place on tissue processor in Formalin 10%, Phosphate Buffered (Part 1090) fixation step.

PROCEDURE NOTES:

1. Nitric acid solutions are not recommended as a decalcification agent following B-5 Fixative Modified, Zinc Chloride fixation.
2. 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 1084) for long-term storage purposes.
3. Alternative uses of B-5 Fixative Modified, Zinc Chloride:
   a. 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.
   b. 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.
   c. Rinse tissue thoroughly in running tap water. Proceed with Formalin 10%. Phosphate Buffered (Part 1090) fixation on tissue processor.
   d. Without the addition of formaldehyde to B-5 Fixative Modified, Zinc Chloride, the quality of tissue fixation may be compromised.
4. 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.
   a. Approximately 100 grams of sodium bicarbonate will neutralize/precipitate zinc from 1 liter of B-5 Fixative Modified, Zinc Chloride.

REFERENCES:

4. Modifications developed by Newcomer Supply Laboratory.