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Chemistry beyond the ordinary

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Super Decal I: Delicate Decal
Super Decal II: Heavy Duty Decal

Catalog #24888 and #24887

Introduction

Polysciences, Inc. Super Decal II: Heavy Duty Decal is a remarkably effective multipurpose decalcifier that can be tailored to suit your specific lab routine. As with most acids, nuclei acids in the cell can become subject to ribonuclease digestion, resulting in a loss of basophilic properties. Careful monitoring should be used with Super Decal II: Heavy Duty Decal to avoid over decalcification. Most decalcification occurs in approximately 4-6 hours or less, depending on the thickness and density of specimens. **Overnight decalcification should be avoided**. Full strength Super Decal II: Heavy Duty Decal is used for hard compact bone, (i.e., femur heads) and specimens such as bone marrow biopsies (core). In either case, the standard procedure should be to check the specimen every 1/2 - 1 hour for mildly calcified specimens and every 1-2 hours for compact bone to determine the endpoint of decalcification. If Immunohistochemistry is to be performed on bone biopsy, Delicate Decal I is strongly recommended.

Techniques for Optimal Decalcification with Super Decal II: Heavy Duty Decal

1. Frequent mild agitation or swirling of the specimen in solution will enhance even penetration and decrease the exposure time of the tissue to the acid solution. This will also minimize over decalcification of the outer tissue or bone before sufficient core decalcifying is achieved.
2. Decalcify the Decal Block Solution will allow you to slow down the decalcification process to suit your specific lab routine.
3. To avoid over decalcification, check specimens at regular intervals for an endpoint. Every 1/2 - 1 hour for mildly calcified specimens and every 1-2 hours for compact bone.
4. Reducing temperature of the decalcifying solution to approximately 20°C will promote histochemical staining in procedures such as H & E, Masson's, Van Gieson's and Azure-Eosin. Delicate Decal I should be used if IHC is to be done on bone marrow biopsy blocks.
5. To remove sediment, Super Decal I and Super Decal II may be filtered if desired without altering its effectiveness.
6. To achieve optimal performance do not reuse Super Decal I or Super Decal II. Since the nature of a decalcifying agent is to release calcium ions from the bone into the acid solution, as the solution becomes saturated with calcium ions the decalcification process will slow down.
7. Addition of an alcoholic solution can aid in preventing undue swelling and hydrolysis of the tissue. It will however slow down the decalcifying agent. An 8:2 ratio of stock Super Decal II: Heavy Duty Decal (8) to 80% alcohol (2) can serve as a standard range for this method.
8. Rinse specimens thoroughly in running tap water after decalcification.

Suggested Times for Decalcification

Bone Marrow Biopsies: 15-30 minutes *(Super Decal I: Delicate Decal strongly recommended)*

Small Cancellous Bone: 2-4 hours

Femur Wedge: 3-5 hours

Mature Bone (1 cm): 4-6 hours

Whole Femur or Teeth: Overnight

Tip: Swirl and check regularly for endpoint. You may dilute Super Decal II: Heavy Duty Decal with tap water to slow the decalcification process.

References

1. A BRIEF ATLAS OF HISTOLOGY: Thomas & Robert Leeson, W.B. Saunder Co. Philadelphia/London/Toronto, 1979.
2. ATLAS OF NORMAL HISTOLOGY: Mariano S.H. diFiore, Lea & Febrger, Philadelphia.
3. LABORATORY MANAGEMENT CONSULTANTS: S. Brown, St. Louis. 1995
4. MANUAL FOR HISTOLOGIC TECHNICIANS 3RD EDITION: Ann Preece, Little, Brown & Co. Boston, 1959, 1969, 1972
5. THEORY AND PRACTICE OF HISTOTECHNOLOGY 2ND EDITION: Dezna Sheehan, Barbara Hrapchak, Battle Press/Columbus Richland

 Active: 24/Dec/2008

RAPID BONE DECALCIFICATION PROCEDURE - RECIPES FOR MICROWAVE STAINING KITS

Summary

This procedure is intended for use as a “stat” alternative to room temperature decalcification of compact bone specimens (e.g. femur head). Due to variability in bone density from one specimen to the next, 2-3 repeats of the final irradiation step may be necessary. It is critical to perform flexibility checks at the stated intervals to prevent over-decalcification and loss of nuclear detail. For optimal effectiveness, the specimens should be secured in a microwavable cassette rack and placed in 300ml of Super Decal II: Heavy Duty Decal in a 600ml microwavable container. Cassettes should be positioned in the rack so that they are located in the mid-portion of the Super Decal II: Heavy Duty Decal during the procedure. Agitation of cassettes between irradiation steps is a critical factor in penetration and performance of the decal solution. Bone specimens used should be no greater than 5mm in thickness.

Note: This procedure is not intended for use on cortical bone (e.g. bone marrows, for bone marrows, use Super Decal I: Delicate Decal at room temperature or 20° C), since cortical bone may be rapidly decalcified in Super Decal II: Heavy Duty Decal Decalcification Solution within 30-45 minutes.

Note: Bone must be fixed prior to decalcification with 10% Neutral Buffered Formalin.

Procedure

1. Trim fixed bone specimen to 2-5mm in thickness, and place in a plastic cassette. Use one cassette per specimen and secure cassette(s) in a microwavable rack.

2. Pre-heat 300ml of Super Decal II: Heavy Duty Decal at 800 watts for 25 seconds, without the rack/cassettes containing the tissue.

3. Place rack/cassettes containing tissue into the preheated Super Decal II: Heavy Duty Decal and agitate rack up and down 20 times. (Use air bubble agitation if available).

4. Place container containing rack/cassettes in the microwave cavity and microwave at 200 watts for 15 minutes.

5. Take container out of cavity in a well-ventilated area or under a fume hood and agitate rack/cassettes up and down 20 times.

6. Place container containing rack/cassettes in microwave cavity and microwave at 300 watts for 15 minutes.

7. Take container out of cavity in a well-ventilated area or under a fume hood and agitate rack/cassettes up and down 20 times.

8. Place container containing rack/cassettes in microwave cavity and microwave at 400 watts for 10 minutes.

9. Take container out of cavity in a well-ventilated area or under a fume hood and agitate rack/cassettes up and down 20 times.

10. Place container containing rack/cassettes in microwave cavity and microwave at 200 watts for 10 minutes.

11. Take container out of cavity in a well-ventilated area or under a fume hood and agitate rack/cassettes up and down 20 times. Determine decalcification endpoint by checking bone using standard methods. If decalcification is not complete, repeat final irradiation step (#10) until desired decalcification endpoint is achieved.

Note: It is critical to check the bone specimen prior to each repetition of final irradiation to prevent over-decalcification and loss of nuclear detail. This procedure should only be used in a laboratory microwave with a ventilation system.

PROCESSING BONE MARROW BIOPSIES FOR HEMATOPATHOLOGY

Principle

When architectural features of both bone and marrow are important in diagnosis, aspirated bone marrow is usually obtained with a sternal puncture needle. Smears are made and the remainder is processed as a biopsy specimen. If IHC, special stains or cytochemical techniques are to bo performed, Super Decal I: Delicate Decal is strongly recommended.

Precautions

- Wear gloves and protective clothing during decalcification procedures.
- Perform procedures under a fume hood.
- **DO NOT MIX FORMALDEHYDE WITH Super Decal II: Heavy Duty Decal, POISONOUS GAS IS LIBERATED.**

Procedure

Bone marrow biopsies are collected and placed immediately into B5 fixative (to which 1.0ml of formaldehyde is added just prior to use.) The time of collection is recorded on the specimen container and at the top of the Pathology requisition. As an alternative to B-5 fixative, Zinc Formalin, Acetic Formalin or Bouins Fixative (Cat. #16045) may be used.

1. Fixation time in B5 fixative is 1-2 hours. Other fixatives penetrate at 1mm per hour.
2. Fill out a gross sheet for bone marrows listing:
    a. Surgical number
    b. Patient's name
    c. Record bone marrow biopsy in the TYPE of TISSUE column
    d. Record the "BM" number in the CASSETTE IDENTIFICATION column
    e. Record the number of pieces of bone marrow in the CASSETTE IDENTIFICATION column
    f. Record the location in the LOCATION column
3. After fixation, wash in tap water for 2-3 minutes (decant used B5 fixative into labeled waste container.)
4. Decalcify specimen in Super Decal II: Heavy Duty Decal solution by pouring filtered Super Decal II: Heavy Duty Decal (approx. 20cc) into specimen container.
5. Decalcification time is 20 to 40 minutes.
6. Biopsy is checked for decalcification by bending to check flexibility.
7. Place specimen into blue cassette labeled with both surgical and bone marrow numbers (decant used Super Decal II: Heavy Duty Decal into proper drain with water flush.)
8. **Wash in running tap water for 1 hour.**
9. Place in 10% buffered formalin until ready to load into processor.

Note: Some biopsies may arrive too late to complete this procedure in its entirety. Store in 10% buffered formalin overnight to be completed the following day. Bone marrow biopsy sections must be air-dried completely before heat-affixation in a 60° C oven for 40 minutes.

References

1. Adapted from the Standard operating Procedures of the Histology Laboratory Department of Anatomic Pathology at EMORY UNIVERSITY HOSPITAL, Atlanta, GA
2. Beard, C., et al., "ACHIEVING TECHNICAL EXCELLENCE IN LYMPH NODE SPECIMENS: AN UPDATE", LABORATORY MEDICINE, Vol. 16, NO. 8, Aug 1985, 468-475
3. Beard, C., Bowling, M., "TECHINICAL FACTORS IN EVALUATION OF LYMPH NODE BIOPSIES", Laboratory of Pathology, Nat'l. Cancer Inst., Nat'l. Inst. of Health, Bethesda, MD
4. Sontoianni, R. Hammami, A., "NUCLEAR BUBBLING: AN OVERLOOKED ARTIFICAT", JOURNAL OF HISTOTECHNOLOGY, Vol. 13, No. 2 June 1990, 135-136

BONE MARROW BIOPSIES

Aspirate

1. Allow aspirate to clot in petri dish.

2. Transfer clot to specimen container filled with 10% neutral buffered formalin.

3. Process as usual.

Bone Core Fixation

1. Immediately fix in Acetic Formalin or Zinc Formailn (Cat. #21516) for a minimum of one hour.

2. Fix overnight if brought in later than 2 hours before end of the work day.

Decalcify first thing in the morning.

Bone Core Decalcification

1. Place fixed core and label in a plastic cassette. **(Super Decal II: Heavy Duty Decal discolors metal)**
   Decalcify in Super Decal I: Delicate Decal, Decalcify the Decal Block Solution (Stat Decal) or Deli-Cal Block Solution for 1 hour.
   Do not leave overnight.

2. Test for decalcification by gently checking for pliability.

3. Rinse in running tap water for approximately 2 minutes.

Processing

1. If biopsy came in afternoon, process with the rest of the specimens.

2. If the biopsy is decalcified in the morning, hand process in 120cc plastic specimen cups, 15 minutes in each container, starting with 70% ethanol or speed process through processor, 15 minutes each station with heat and vacuum, starting with the first dehydrant station.

Microtoming

1. Cut both aspirate and bone cores at 2 microns.

2. Place 3 levels on one slide. Cut an extra slide of the last level and set aside on the back of the water bath in case a special stain is requested.

Staining

1. Hematoxylin approximately 5 minutes. Bone core 1 minute (check under the microscope before counterstaining).

2. Eosin for 10 dips, dehydrate, clear and coverslip.

Note:
Over decalcification will result in poor or indifferent histological detail and staining characteristics. Less than one hour is usually not sufficient.

Reference
1. Adapted from Becky Scholes, HTL,MT(ASCP) H.I.S.T.O., The Official Newsletter of the Iowa Society for Histotechnology, Tech Tips

TECHINIQUES FOR PREPARTATION OF BONE MARROW SPECIMENS IN PARAFFIN AND PLASTIC

Handling of Bone Marrows

Aspirated Marrow Units

1. Add about 5ml of marrow aspirate into a specimen collection tube containing 0.07ml 15% EDTA and mix the contents quickly and thoroughly.
2. After arriving in the laboratory, the contents of the specimen collection tube are filtered through either templefiber or Histowrap recovering only units.
3. The units are wrapped in templefiber or Histowrap and placed in a bottle containing working B-5 fixative. The units are fixed for 1 hour.
4. The wrapped units are placed in a plastic processing cassette **(do not use metal)** and stored in 70% ethyl alcohol until processing.

Needle Biopsy of the Marrow

1. Biopsy material is placed in a bottle working B-5 fixative.
2. Fix biopsy for a minimum of 30 minutes; 1 hour preferable.
3. After fixation, rinse the biopsy in tap water.
4. The specimen is placed in Super Decal II: Heavy Duty Decal decalcifying solution for 15-30 minutes.
5. When the decalcification is completed, the specimen is wrapped in lens paper, placed in a plastic bag processing cassette (no metal) and washed in running tap water for 30 minutes.
6. Process as usual.

Reference

1. Adapted from Karen Noe, B.S., HTL (ASCP), Muhlenberg Regional Medical Center, Plainfield, NJ 07060

METHOD TO FACILITATE SECTIONING INCOMPLETELY DECALCIFIED BONE

Bone Biopsies are sometimes difficult to obtain and uncomfortable for the patient. Therefore, incomplete decalcification before processing can create problems if the bone sections cannot be achieved without loss of tissue and require re-decalcification, which takes precious time.

Ideally, the specimen should not be processed until decalcification is complete. Sections of bone that have been hurriedly processed and fail to section because of incomplete decalcification may be placed in Stat Decal Solution for a short period of time (this will vary the density and size of the bone), washed in ammonia water, rechilled and sectioned in the same day without having to remove from the block and reprocess. Small pieces of bone such as bone marrow biopsies take only a short soak of about 30 minutes and will section easily. Larger pieces also will section but take a longer soak. The depth of effectiveness is minimal and if levels are needed, the block may have to be reintroduced to the decal solution more than once.

Tip: If the block is “crunchy” after facing, soak the block either on plain ice if the specimen is not too hard or in Decalcify the Block Solution or Super Decal II: Heavy Duty Decal if more harsh treatment is necessary.

Reference

1. Adapted from Shirley A. Powell, HT (ASCP) HTL, Technical Director, Histopathology Laboratory, MERCER UNIVERSITY SCHOOL OF MEDICINE
MICROTOME The Official Newsletter of the Georgia Society for Histotechnology, Volume XV Summer 1993, No. 3

ANIMAL TISSUE TECHNIQUES

Decalcification

Calcium deposits may be so heavily concentrated in the tissue that they may interfere with sectioning and result in torn sections and nicks on the knife edge. If deposits are sparse, overnight soakings of blocked tissue in water will soften the deposits sufficiently for sectioning. Heavy deposits may be removed by any of several methods, but do not leave tissue in any fluid longer than necessary.

If any doubt arises about the completion of decalcification, check for calcium by the following method:

To 5ml of the solution containing the tissue add 1ml of 5% sodium or ammonium oxalate. Allow to stand for 5 minutes. If precipitate forms, decalcification is not complete. A clear solution indicates it is complete. We do not recommend sticking needles in the tissue to check hardness as this may damage cells.

Old bones cut down to one centimeter in thickness, if possible, require a six hour treatment: small and young pieces, only one to two hours. Teeth will require overnight and up to eighteen to twenty-four hours. Do not over decalcify; this detracts from the staining quality. Decalcifying may be followed by a brief washing in water but this is not necessary. Fixation and decalcification may be combined in a mixture of one part undiluted formalin with nine parts Super Decal II: Heavy Duty Decal.

Precautions

The combination of Super Decal II: Heavy Duty Decal and formalin is discouraged, but should always be done under a fume hood to ensure the removal of potentially harmful vapors. Always follow the suggested directions for use.

Reference

1. ANIMAL TISSUE TECHNIQUES, 4th EDITION: Gretchen L. Humason, WH Freeman & Co. Gordonsville, Virginia 1979.

Ordering Information

Catalog #	Description	Size
24888	Super Decal I: Delicate Decal	500ml, 1 liter, 6 x 1 liter
24887	Super Decal II: Heavy Duty Decal	500ml, 1 liter, 6 x 1 liter

Adjuncts / Additional Products

Catalog #	Description	Size
18606	Aqua-Poly/Mount	20ml, 100ml, 5 X 20ml
09859	Eosin Y, 0.5% alcoholic solution, Acidic	500ml, 1000ml, 3.75 liters
17269	Eosin Y, 1% alcoholic solution, Non-Acidic	500ml, 1000ml, 3.75 liters
24606	Flash Dip - FNA / H.Pylori Stain Kit	250ml, 500ml
22363	Fungi-Fluor® Pneumocystis Kit (U.S. & outside Europe)	1 kit
22363E	Fungi-Fluor® Pneumocystis Kit (European)	1 kit
24205	Gomori's Trichrome Stain Kit	1 kit
24245	Harris Hematoxylin, Acidified (mercury-free)	500ml, 1000ml
19562	PolyFin® Paraffin	1 case (8 x 1kg), 1 keg (15kg)
22247	Poly-L-Lysine Coated Microscope Slides	1 box
08381	Poly-Mount	120ml, 940ml
24119	Poly-NoCal End Point Determination Kit	1 kit
24199	Prussian Blue Reaction for the Demonstration of Iron	1 kit
24216	Tissue Tack Microscope Slides™ Plus(+) Glass Slides	1 box
08711	Wright-Giemsa Stain Solution	470ml
21516	Zinc Formalin Fixative, pH 6.25	3.75 liters

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