Electron
Microscopy
Sciences

INSTRUCTIONAL MANUAL
CATALOG
61700-01
61701-01
61704-01 through 61704-06
Series: Well Bar Sets

Cryoembedding Techniques

Electron Microscopy Sciences
P.O. Box 550, 1560 Industry Road, Hatfield, PA 19440
www.emsdiasum.com
Toll Free: **1-800-523-5874**
sgkcck@aol.com
Tel: 215-412-8400 ♦ *Fax:* 215-412-8450

Introduction

The following directions give you a basic outline of cryoembedment and explains a few different cryoembedding techniques that make it easier to obtain proper specimen orientation and successful embedding in a cryostat, using the components included in the Well Bar Set. Store components at cold temperatures so that specimens freeze quickly, most in 20 to 60 seconds, depending upon their size and the selected freezing technique. This significantly reduces turn-around time.

Basic maintenance is also addressed, after the descriptions of the different cryoembedment techniques.

Basic Directions for Cryoembedding

In general, reduce freezing artifacts by keep embedding medium in the refrigerator. Also, pre-chill the specimen and dispensing slides on a piece of metal.

Orienting the Specimen *(More detailed techniques for embedding the specimen are explored after these basic directions)*

- Orient specimen so any fat is the last part to be cut. Since fat freezes fully at much lower temperatures than most other parts of the specimen, when the knife cuts into fat, the fat can pull out the tissue or smear and ruin the rest of the specimen.
- Orient specimen so the desired aspect is perpendicular to the blade, and not the first or the last to be cut.
- Specimens that have varying consistencies and/or toughness should not be placed so the various differences will be cut at the same time.
- Make sure there is a uniform layer of medium on the dispensing slide, which will result in the flattest possible plane.
- Make sure there is nothing between the chuck and chuck holder so that the chuck is always flush to the holder

Handling Numerous Specimens

- Prepare all dispensing slides and keep them in order.
- Fill wells.
- Label chucks after placing over the well. (If chucks are thoroughly cold, freezing time is very quick - possibly 15 seconds.
- Place wells on cold surface while you prepare more blocks.
- First set of blocks should be ready to be tapped out.

Filling the Well with Medium

- After placing the specimen in the well, fill until medium forms a meniscus that protrudes a bit above the level of the well. **NOTE:** If the well is under filled, the medium will not fill the channels and the chuck might not adhere properly and could come off.

Parallel Faces

- Place chuck flush over the well so its surface is parallel to the well base, thus ensuring the chuck and block face are parallel.
- If you experience problems getting the two parallel, try using a chuck larger than the well to create parallel surfaces. **NOTE**: Parallelism ensures that the entire block face will cut at the same time, which is important for the smaller specimens.
- When cutting, if top or bottom of specimen cuts first, carefully adjust the vertical angle of the block face.

Tightening the Screws

- When mounting the chuck, clamping in the blade, or any mechanism that holds them, be sure to tighten screws and clamps securely. A few microns of movement can cause thickness variations when cutting tough, leathery tissue.

Icy Samples

- Specimens with a high liquid/water content become brittle and shatter when cut. If this happens, pick the pieces up with a shallow container, mix in some medium and put them in the well.

Removing Block from Chuck

- Hold block firmly and tap block sharply towards you.

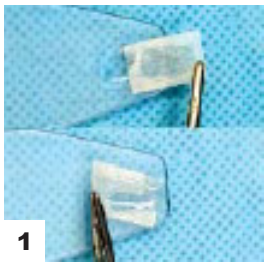
Cleaning the Chuck

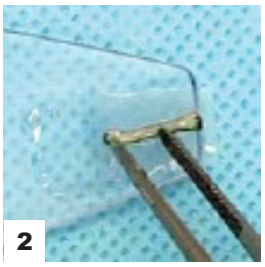
- To remove specimen from the chuck, dip it in to formalin for a few seconds and then, with pointed forceps or a thin screwdriver, pry it off before it melts.

Paper Cryoembedment

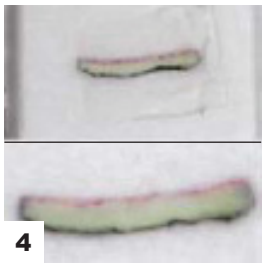
This technique is especially good for those difficult-to-work-with specimens. It can be used to maintain orientation of delicate or flimsy specimens and also for arranging multiple specimens so they will remain in the same plane for sectioning.

Single Specimen Section


1


2


3


4

Untrimmed block

Trimmed block

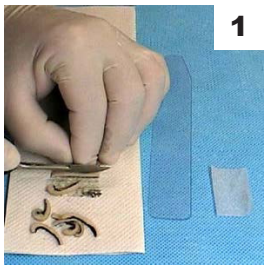
Technique

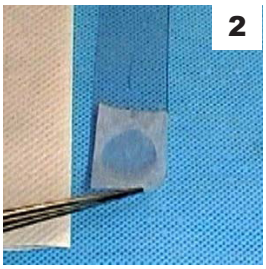
1. A drop of embedding medium is placed on the dispensing slide. Soak a small piece of lens paper in embedding medium and flatten it to the dispensing slide. Add a drop of medium on both sides. Press out excess with forceps.
2. Place the specimen on the lens paper and orient appropriately. Allow an end of paper to overhang on the edge of the dispensing slide.
3. Touch the lens paper to the cold floor of embedding well and gently pull dispensing slide away. Specimen will remain correctly positioned on the lens paper.
4. Trim through the paper on the trimming portion of the blade and then move to a clean, sharp portion to section the specimen.

Result

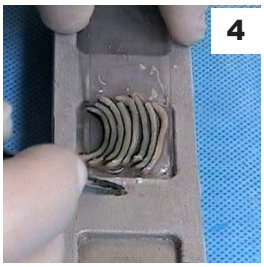
Photomicrograph

Multiple Specimen Sections


1


2

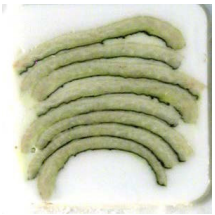

3


4

Technique

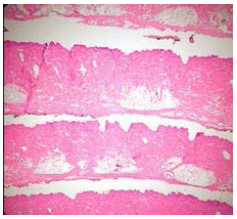
1. Start with dry lens paper cut to size of specimen and cut tissue into strips. Lightly coat specimen strips with embedding medium.
2. Place a drop of embedding medium on dispensing slide and then wet both sides of paper.
3. Place specimen strips on paper on their edge, close together, and leave a 2-3 mm tab of paper to overlap edge of dispensing slide.
4. Press overlapping paper tab to well floor and pull the slide away. Fill well with medium.

Results



Trimmed block

Stained section on slide



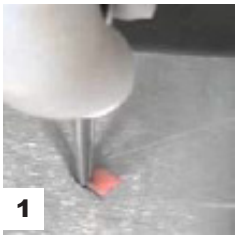
Photomicrograph

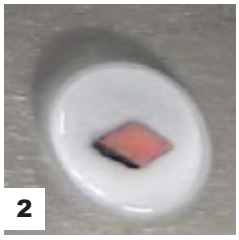
Special Notes

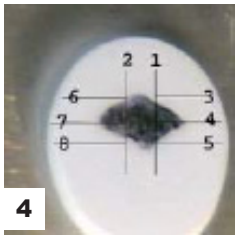
- Completely wet the paper on both sides, including the tab hanging over the edge of the dispensing slide.
- Press any excess embedding medium or bubbles out from under the paper with the side of the embedding forceps.
- To pull paper evenly off the slide, touch the wetted tab *along its entire width*.
- The lens paper cuts away easily and does not interfere with the section. Fill any defects that may arise.
- Once the paper is adhered to the well floor, trim away any excess paper.

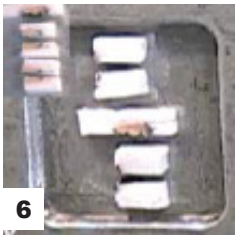
Frozen Block Cryoembedment

Specimens are embedded and frozen in their entirety, then mapped and cut into firm, flat pieces. While still frozen, flat pieces are then embedded on edge. This technique is perfect for those "imperfect" specimens that are harder to handle because they may be tubular, angular, curled, or flimsy. It is also very useful for margin resections.









Technique

1. Place tissue on thin film of embedding medium and lay specimen face down on freezing griddle.
2. Cover specimen with a layer of embedding medium.
3. Cover specimen with appropriate elevated freezing block.
4. When completely frozen, map the specimen.
5. Cut embedded block into pieces on the cold cutting board, keeping pieces cold on the adjacent metal surface. (Photo #5 shows the central section; inset shows a longitudinal margin.)
6. Place cut pieces face down (photo #6 inset shows pieces face up) in the embedding well and freeze using the procedure described under ***Face Down Cryoembedment***.

Results

Trimmed block

Stained section on slide

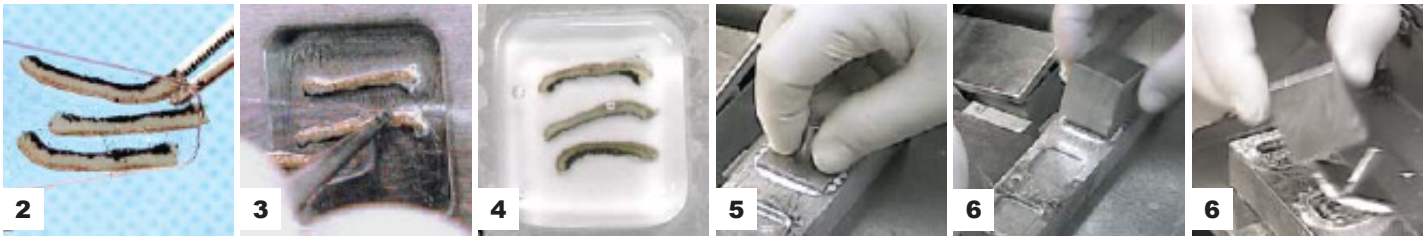
Photomicrograph Note: Sections are repositioned in photograph

Special Notes

- Flatten curling or rubbery tissue by touching edge of tissue to freezing griddle and gently pull/stretch tissue to desired position.
- Approximate freezing times: 18 mm well – 20 secoonds; 24 mm well – 35 seconds; 30 mm well – 60 seconds
- The block should cut like hard ice cream.
- If cut piece flies out, it is too hard. Set block aside for a few moments and cut again. Repeat as necessary.
- When cutting longitudinal margins push the blade toward the medium and make sure the tissue surface is well frozen to the medium to prevent dislodging.

Face Down Cryoembedment

With this technique, it is possible to embed any type of specimen flat and with perfect orientation, whether it be single or multiple pieces, liquid or solid, large or small.



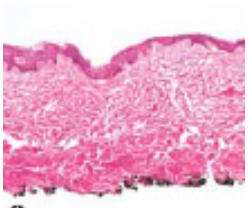
Technique

1. Apply a thin layer of embedding medium to tip of slide.
2. Precisely orient the specimen(s), face down, on the plastic dispensing slide. From underside, check for desired tissue alignment.
3. Touch the edge of a specimen to the cold base of the embedding well, gently withdrawing the dispensing slide while positioning the specimen. Repeat as necessary.
4. Slightly overfill the well with embedding medium.
5. Place a chuck over the well.
6. Place an over-chuck freezing block over the stem of the chuck.
7. Remove freezing block and tap the chuck stem when freezing is complete (usually 20 to 60 seconds) to remove the embedded block from the embedding well.

Results

Trimmed block

Stained section on slide



Photomicrograph

Special Notes

With the Face Down technique, sometimes there maybe imperfections in the block face that will interfere with the embedding medium fully adhering to the specimen, thereby making the tissue curl away from the medium. To help prevent this situation, do the following:

1. Place a drop of embedding medium on the chuck face.
2. Press the face to a flat, freezing surface – a cryostat stage any of the freezing apparatus.
3. Tap the chuck to remove it.

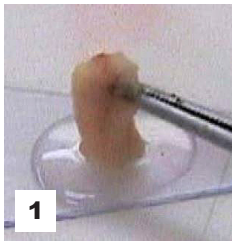
Repairing Imperfections in the Block Face

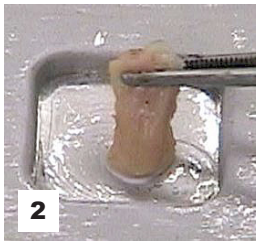
When curling of the specimen happens, add embedding medium in this manner:

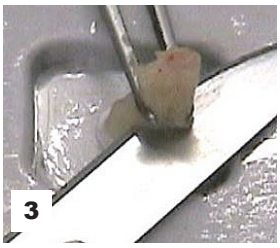
1. Place drop of embedding medium on chuck face.
2. Press face to a flat freezing surface (cryostat stage or any freezing apparatus.
3. Remove chuck with a tap.

Difficult-to-Trim Specimens

This technique can be applied to anything that is difficult to neatly trim to 3 mm. The freezing steel holds the tissue while the well bar surface acts as a guide for the scalpel.



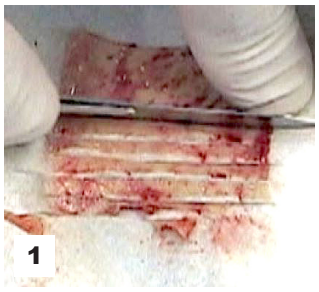


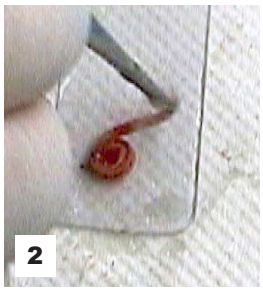


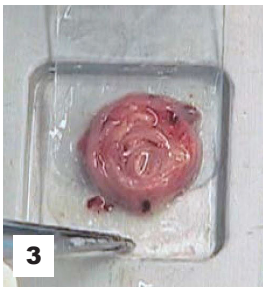
1. Place the end of the tissue in a drop of embedding medium.
2. Touch the margin to the well floor to which it will adhere.
3. Hold the tissue with a forceps and cut it off with a scalpel blade at the level of the well bar surface. Make sure blade is sharp and use a gentle slicing motion with the scalpel.
4. Fill the well and apply the chuck.
5. Continue with freezing process.

Rolled Technique for Thin Membranes

This technique is good for extremely thin specimen that need to stand on end to study desired ROI (region of interest).







1. Cut membrane into 3mm wide strips. Wet with embedding medium.
2. Place paper on slide as above. Start by rolling a strip on the paper to form the center. Add additional strips by wrapping them around the central core.
3. Touch tab paper to the well floor. Pull paper and rolled specimen off the slide onto the well floor.
4. Continue with freezing process.

General Maintenance for Equipment

Be sure to clean all parts of the system right after using.

Well Bar: Use gauze dampened with alcohol and wipe. **NOTE:** DO NOT leave any alcohol residue or the next specimen will not adhere when put into well.

Chuck: Run chuck under water while brushing its teeth. Dry with a towel. Replace in chuck bin.

Dispensing Slide: Rinse with water, then dry by pulling slide through clean lab towel. NOTE: If medium is left to dry on dispensing slide, it will be harder to clean. **NOTE:** DO NOT use abrasive cleaners on the vinyl slides as they scratch easily.

Electron
Microscopy
Sciences

For any questions or for ordering information,
please contact Customer Service at
1-800-523-5874

Thank you for choosing
Electron Microscopy Sciences!

www.emsdiasum.com
sgkcck@aol.com

Tel: 215-412-8400 ♦ *Fax:* 215-412-8450

Electron Microscopy Sciences
P.O. Box 550
1560 Industry Road, Hatfield, PA 19440