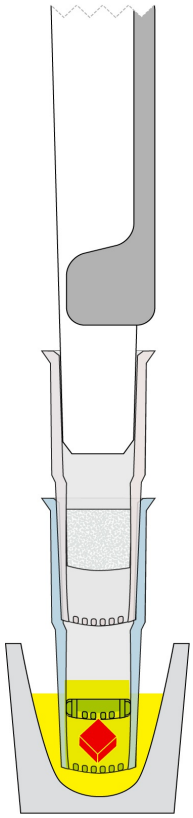
Preparing Specimens with mPrep/s™ Capsules

mPrep/s™ capsules offer an end-to-end system for processing, embedding and archiving TEM or SEM specimens that:

- Accomplishes fluid reagent processing and embedding in a single capsule.
- Allows easy and sure specimen orientation prior to or during processing.
- Gives consistent, high-quality results with almost any processing protocol.
- Decreases manual specimen handling. Cuts labor and specimen damage.
- Reduces reagent consumption by over 75%.
- Provides continuous specimen labeling for GLP compliance.

Summary of mPrep/s™ device function:

- The mPrep/s capsule holds the specimen within a pipette-like tube, entrapped between 300 µm mesh screens.
- The capsule attaches to ordinary lab pipettors, which deliver reagents and embedding resins to specimens by pipette action (see drawing on right). Capsule volume is small to assure efficient use of reagents.
- If desired, specimens may be oriented within the capsule. Among several available methods is a novel pinching method ideal for long, thin and delicate specimens, such as neural and plant tissues or fibers.
- Without further manual handling of the specimen, embedding takes place in the mPrep capsule. No embedding mold is required. TEM specimen blocks are sectioned without requiring removal from the capsule.
- Barcode and/or alphanumeric labels on capsules provide a unique sample ID for each specimen, facilitating sample tracking from the point of acquisition through reagent processing, sectioning and storage.
- Specimen blocks are stored in provided boxes, which can also hold mPrep/g™ capsules containing grids associated with the specimen blocks.



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INSTRUCTIONS

Equipment Needed

- **mPrep/s capsules** — each capsule is pre-loaded with a removable screen.
  - *Extra screens are supplied, in case of loss or need for experimental modification.*
- **Insertion tool**
- **Labels** — a set of four identifying labels is supplied with each capsule.
- **P200 Pipetman®** or equivalent laboratory pipettor capable of delivering 10 - 200 µl
  - *mPrep/s capsules are designed to fit most brands of single- or multi-channel laboratory pipettors. Instructions are provided here for an 8-channel Gilson P200N Pipetman. There may be slight differences if using other lab pipettor devices.*
- **mPrep/f couplers** — to protect the pipettor from unintentional aspiration of reagents.
- **reagent reservoirs** — reagent reservoirs (or other reagent vessels) for holding processing chemicals must have suitable chemical resistance.
  - *HDPE (high density polyethylene), PE (polyethylene) or PP (propylene) are usually suitable.*
  - ***Warning:** Avoid commonly available polystyrene reagent reservoirs, which are not compatible with most embedding solvents and resins.*
- **mPrep/bench** — silicone 96-well plate to hold mPrep/s capsules during resin polymerization; also convenient to retain fluid-filled mPrep/s capsules at other times.

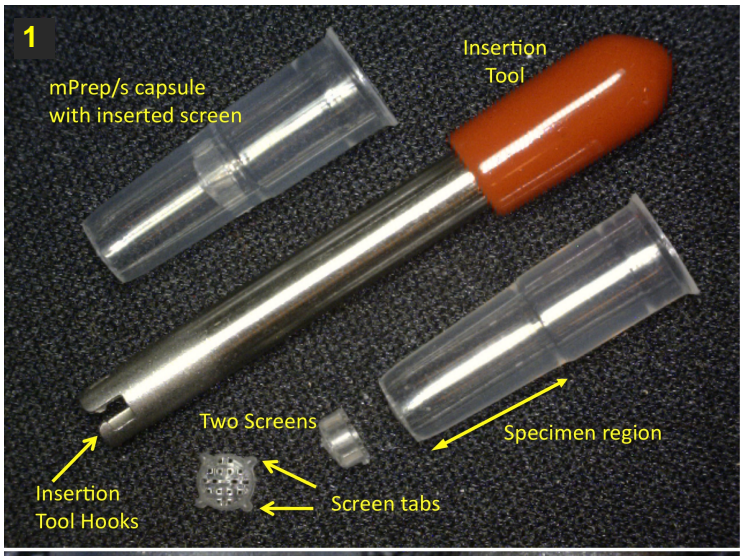
Optional Equipment

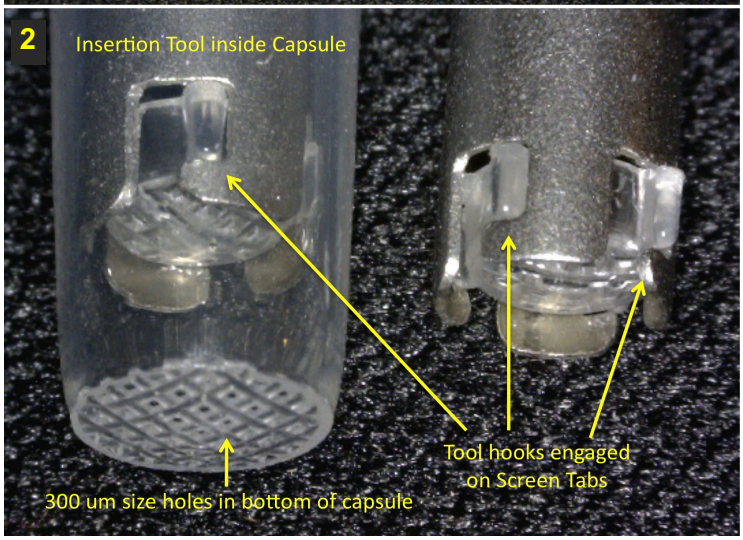
- **mPrep/s Workstation** — a fast, easy way to prepare specimens and insert them into mPrep/s capsules with orientation.
- **96-well plates** — may be used to hold mPrep/s capsules.
  - ***Warning:** Use only polyethylene, polypropylene or other chemically resistant plates.*
- **mPrep CPD Immersion Holder** — holds mPrep/s capsules in critical point drying apparatus

mPrep/s Components and Basic Use

The three basic mPrep/s components are CAPSULE, REMOVABLE SCREEN and INSERTION TOOL (fig. 1). Using mPrep/s is simple:

- Specimens are placed into the **capsule**. The **removable screen** is positioned to entrap the specimen between capsule bottom and the screen.
- Both **capsule** and **screen** have 300 µm holes to allow reagents to flow to the specimen (figs. 1 & 2). Liquid reagents — including viscous embedding resins — are easily drawn into the capsule by use of a pipettor.
- The friction-fit **screen** can be placed at any location in the "specimen region" (identified in figs. 1 & 5) to accommodate specimens of various shapes and sizes.
- Specimen orientation can be easily accomplished at this time, if desired, reducing later effort and materials.
- The **insertion tool** has hooks to engage tabs on the **screen**, enabling easy insertion or removal of the screen from the capsule.
  - *Engage the hooks by turning slightly to the right, and disengage by turning left.*
  - *Fig. 2 shows a screen held within an insertion tool inside a capsule, and a second screen in an insertion tool that is not inside a capsule.*





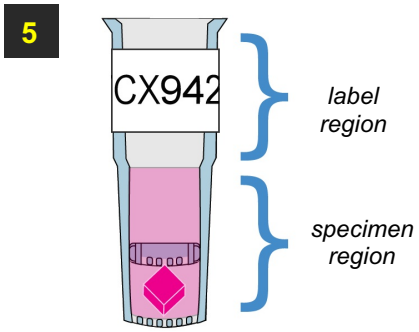
Loading specimens into capsules — *without* orientation

When specimen orientation is not required, the specimen is simply placed in an mPrep/s capsule and then entrapped by the screen. The procedure is as follows:

1. Remove the pre-loaded screen from the capsule:
   a. Slide the insertion tool into the capsule and engage its hooks onto the screen tabs.
      - *First-time users may find magnification helpful for this step.*
   b. Slightly rotate the insertion tool clockwise to fully engage the hooks on the tabs.
   c. Remove the insertion tool with the screen "loaded."
      - *If your capsule did not contain a pre-loaded screen, then a screen must be loaded into the insertion tool prior to step 2. The method is explained below in Additional Techniques.*
2. Place the specimen into the bottom center of the mPrep/s capsule.
   - *This may be done with the capsule sitting in an appropriate fluid to keep samples hydrated.*
   - *Use a 96-well plate (fig. 3), the mPrep/bench silicone rack or the side wells of the mPrep/s Workstation for this purpose.*

3. Slide the insertion tool with the "loaded" screen into the capsule.
   - *The screen may be placed at any location in the lower half of the capsule to accommodate specimens of varying size. (See "specimen region" in figs. 1 & 5.)*
4. Release the screen by rotating the insertion tool counter-clockwise to disengage its hooks from the screen tabs. Then pull the insertion tool out.
   - *The screen may be removed and re-inserted at any time prior to polymerization of embedding resin. Be sure to thoroughly clean any reagents or resin from the insertion tool.*

5. Label the mPrep/s capsule with the supplied labeling system. Corresponding labels with the same unique specimen identity are provided for lab notes, storage containers, etc.
   - *Four pre-numbered labels (fig. 4) are provided for each capsule: a round label for bar code or alphanumeric; and three rectangular labels, which can include both alphanumeric and bar code.*
   - *Custom labels may be requested from Microscopy Innovations.*
   - ***Warning:** Avoid placing a label on the exterior of the capsule's specimen region (fig. 5), as this is where the capsule may need to mate with other capsules when stacking (see Additional Techniques). It also limits exposure of the label to reagents during subsequent processing.*

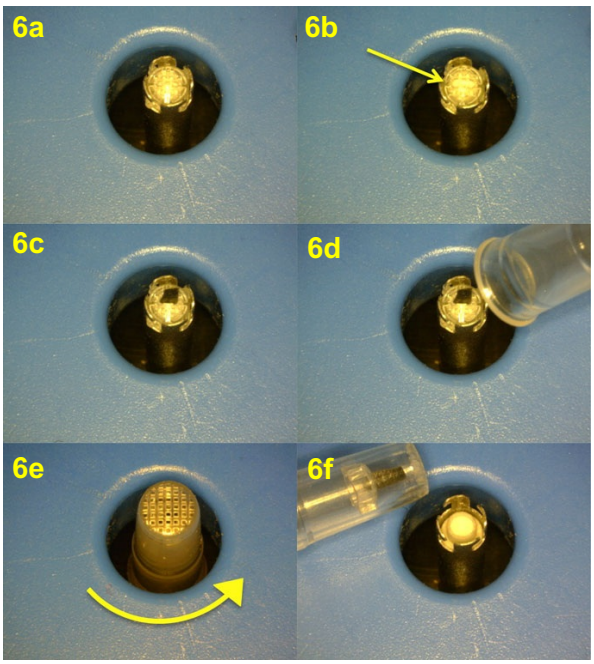


Loading specimens into capsules — *with* orientation

A unique capability of mPrep/s capsules is easy orientation of specimens. Orientation can be established when loading the capsules and will be maintained throughout fluid processing and embedding steps. The capsule itself becomes the embedding mold, eliminating additional handling of the specimen.

The mPrep/s Workstation provides the easiest way to load and orient specimens in mPrep/s capsules. (A more difficult and limited technique using only the insertion tool is presented in Additional Techniques, below.) The instructions with the workstation are as follows:

1. Remove the pre-loaded screen from the capsule:
   a. Place an mPrep/s capsule with pre-loaded screen over the insertion tool mounted in the workstation.
      - *Note that the insertion tool is mounted vertically, so that the screen will be held horizontally at the top of the tool.*
   b. Slide the capsule onto the insertion tool and engage the hooks onto the screen tabs.
   c. Slightly rotate the capsule clockwise to fully engage the hooks on the tab.
   d. Lift the capsule straight up leaving the screen loaded on the insertion tool.
      - *If your capsule did not contain a pre-loaded screen, then a screen must be loaded into the insertion tool prior to step 2. The method is explained below in Additional Techniques.*
2. Orient a specimen on the screen using one of the following methods:
   a. Screen Pinch method:
      i. Begin with screen loaded on the insertion tool (fig. 6a).
         - *Check that screen tabs are fully engaged on the insertion tool by rotating the screen clockwise.*
      ii. Spread open the slit in the center of the screen by pressing the workstation actuator lever. (The open slit is indicated with the arrow in fig. 4b.)
         - *The actuator pushes a rod under the screen to flex open the slit.*
      iii. Slide the "back" end of the specimen into the slit between the center "teeth" and release the actuator to close the slit. This pinches the back end of the specimen, holding it in the desired orientation (fig. 4c).



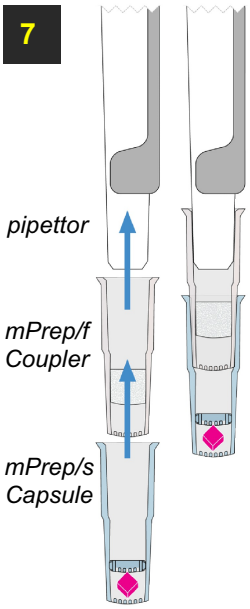
   b. Compression method:
      i. Place specimen onto the screen in the desired orientation. In the next step it can be entrapped to hold that orientation if light compression is applied when you slide the mPrep/s capsule down over the screen. The specimen will be held between screen and capsule.
   c. "Glue" method:
      i. Place specimen on the screen and temporarily "glue" it in place with a suitable material for your specimen. Choose a "glue" that can later be dissolved or embedded.
         - *Potentially suitable "glues" are: low melting temperature agar; cyanoacrylate "super" glue; glycerol or sugar solutions which hold specimens by virtue of their viscosity.*

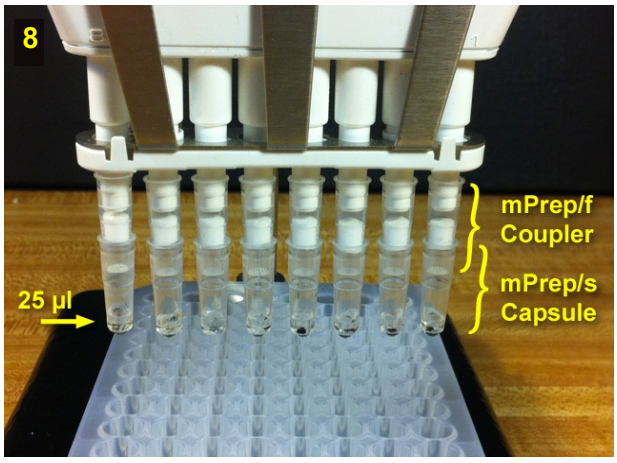
3. Load the capsule with the specimen-containing screen:
   a. Slide an empty mPrep/s capsule straight down over the specimen-containing screen (fig. 6d).
      - *Observe from the side to adjust how far the screen is being inserted into the capsule.*
      - *If desired, lightly compress the specimen between capsule and screen to hold its orientation.*
   b. Rotate the capsule counter-clockwise (arrow in fig. 6e) to release the screen from the insertion tool tabs.
   c. Pull the capsule straight up. The specimen is now loaded in the capsule and ready for processing (fig. 6f).
4. Set aside the specimen-containing capsule while you load additional capsules by repeating steps 1-3.
   - *As required, place each capsule in water or buffer to keep samples hydrated.*
   - *The side wells of the workstation are designed for this purpose. You may also use a 96-well plate (figs. 3 & 9) or the mPrep/bench silicone rack.*
5. Label the mPrep/s capsule with the supplied labeling system. Corresponding labels with the same unique specimen identity are provided for lab notes, storage containers, etc.
   - *Four pre-numbered labels (fig. 4) are provided for each capsule: a round label for bar code or alphanumeric; and three rectangular labels, which can include both alphanumeric and bar code.*
   - *Custom labels may be requested from Microscopy Innovations.*
   - ***Warning:** Avoid placing a label on the exterior of the capsule's specimen region (fig. 5), as this is where the capsule may need to mate with other capsules when stacking (see Additional Techniques). It also limits exposure of the label to reagents during subsequent processing.*

Preparing the pipettor and attaching capsules

Users often process multiple specimens at one time, so we recommend using an 8- or 12-channel pipettor. Photos show an 8-channel P200N Pipetman® pipettor. A single-channel pipettor may also be used. Here are the steps to prepare the pipettor and attach capsules:

1. Set the multichannel P200N Pipetman (or equivalent pipettor) to a sufficient volume to ensure that the specimen will be fully immersed and there is adequate reagent for reaction.
   - *Specimens smaller than 1 mm in all dimensions require a minimum volume of 10 µl.*
   - *A more typical amount is 25 µl, which is more than sufficient for most specimens and all reagents except resin.*
   - *Use 100 µl for resin since it is difficult to control volume with this viscous material.*
   - ***Note:** The maximum capacity of an mPrep/s capsule is 150 µl.*
2. Connect an mPrep/f Coupler™ (or empty mPrep/s capsule) to each channel of the pipettor, as shown at right in figure 7.
   - *This is recommended to prevent accidentally aspirating reagents into the pipettor. The mPrep/f Coupler has a filter, which provides additional protection to the pipettor.*
   - *Couplers may be reused unless they become clogged or contaminated.*
3. Connect each specimen-containing mPrep/s capsule to one of the mPrep/f couplers (or empty capsules), as shown in figure 7.
   - *Fig. 8 shows eight specimen-containing mPrep/s capsules properly mounted on the pipettor using mPrep/f couplers.*
4. Confirm that pipettor volume is sufficient to cover the specimens, as follows:
   a. Remove any fluid that may be in the capsules:
      i. Press the pipettor plunger to the first stop (dispense position)
      ii. Pause for ~1 second
      iii. Press the plunger to the second stop (purge position).
      iv. Release plunger slowly.
   b. Begin the test of fluid volume by pressing the pipettor plunger to its first stop position.
   c. Insert mPrep/s capsule tips into buffer, water or other liquid appropriate for your specimen.
   d. Allow the plunger to slowly move up to aspirate fluid into the capsule.
   e. View the fluid level in the capsule to determine that specimens are covered.
      - *The pipettor in fig. 8 is set to 25 µl, which adequately covers the specimens. The capsules have just been filled with buffer from a 96-well plate.*
   f. If the volume is correct, begin reagent processing as described below.
   g. If the volume is not correct, then adjust as needed and repeat all of step 4, above. Once proper volume is determined, it need not be readjusted until processing with viscous resin.



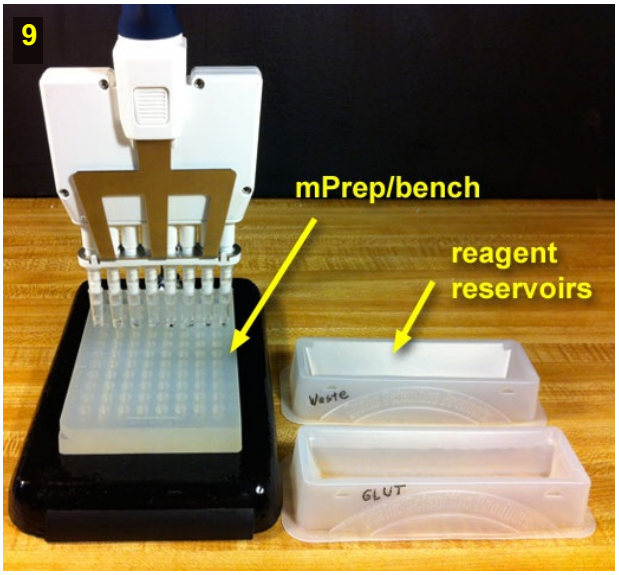


Fluid reagent processing for TEM embedding

The following generic instructions can be adapted for most chemical protocols. Please carefully note the general directions and cautions as you adapt your current protocols to the efficient mPrep System methodology.

1. General directions
   - Aspirate and dispense slowly. When aspirating, always keep the tips of specimen-containing capsules fully immersed in reagent.
     - *This prevents bubbles from forming and ensures specimens will be fully covered by reagent.*
     - *If any bubbles form, slowly dispense and purge the reagent; then re-aspirate more slowly.*
   - High viscosity reagents — such as resins — require very slow aspiration and dispensing. Additionally, these viscous reagents require pipettor volume set at 100 µl to ensure sufficient filling of capsules. Viscous reagents are difficult to pipette accurately.
   - Hold the pipettor in a vertical position during incubations. A lab stand accessory offers a convenient way to do this. It may be desirable to cover the end of the capsules during incubations:
     - *For short incubation times, rest the mPrep/s capsule tips on Parafilm (or equivalent).*
     - *For long incubations — or if evaporation of volatile solvents is a problem — use the mPrep/bench™ silicone rack to provide a tight seal around capsules. Or you may wrap the bottom of the capsules in Parafilm.*
   - **Caution:** Use only reagent reservoirs with suitable chemical resistance. Polyethylene or polypropylene containers are appropriate for most solvents and resins.
     - *Fig. 9 shows Microscopy Innovations' HDPE reservoirs.*
   - **Caution:** Dispense all reagents to a suitable waste, using an appropriate container and safe handling techniques. The term "waste" in the instructions below refers only to a suitable waste.

2. Primary fixation
   a. Pour sufficient fixative (such as buffered glutaraldehyde) into a reagent reservoir.
   b. Empty capsules of prior reagent or other fluid, if necessary (step 4a of "Preparing the Pipettor," above.)
   c. Depress pipettor plunger to the first stop.
   d. Insert capsules into the fixative and slowly aspirate into the mPrep/s capsules.
   e. Wait the appropriate amount of time to ensure reaction, keeping the pipettor upright.
      - *If desired, when incubation begins any fresh reagent remaining in the reservoir may be returned to storage for later use.*
      - *Fig. 9 shows pipettor held on a lab stand with mPrep/s capsules resting in the mPrep/bench rack during fixation.*
   f. Slowly dispense used fixative from the capsules to waste.



3. Rinse
   a. Pour sufficient rinse solution into a reagent reservoir.
   b. Empty capsules of prior reagent or other fluid, if necessary.
   c. Depress pipettor plunger to the first stop.
   d. Insert the capsules into the rinse and slowly aspirate into the mPrep/s capsules.
   e. Wait the appropriate amount of time to ensure reaction, keeping the pipettor upright.
      - ***Important! Rinse at least 2 or 3 times.*** *Capsule volume is intentionally small to minimize reagent consumption Therefore, one exchange with water or buffer may be insufficient. Multiple rinses can be accomplished easily and rapidly.*
   f. Dispense used rinse from the capsules to waste.

4. Secondary fixation
   a. Repeat the directions for primary fixation.

5. Secondary rinse
   a. Repeat the directions for first rinse.

6. Serial dehydration in ethanol or other solvent
   a. Pour sufficient dehydration solvent into a reagent reservoir.
   b. Empty capsules of prior reagent or other fluid, if necessary.
   c. Depress pipettor plunger to the first stop.
   d. Insert the capsules into the dehydration solvent and slowly aspirate into the mPrep/s capsules.
   e. Wait the appropriate amount of time to ensure reaction, keeping the pipettor upright.
      - *It is important to place the capsule tips in the mPrep/bench or rest them on Parafilm to reduce evaporation of solvents.*
      - *If desired, when incubation begins any fresh reagent remaining in the reservoir may be returned to storage for later use.*
   f. Slowly dispense used dehydration solvent from the capsules to waste.
   g. Repeat steps 6a-6f though the entire serial dehydration series, such as 50%, 70%, 90%, and several exchanges of 100% ethanol.

7. Intermediate solvents (such as propylene oxide or acetone)
   a. Repeat the directions for serial dehydration solvents.
      - ***Note:*** *Two or more exchanges of the intermediate solvent are recommended due to the small volume of the mPrep/s capsules.*

8. Resin infiltration for TEM embedding
   a. Reset pipettor volume to 100 µl.
   b. Pour sufficient resin-intermediate solvent (such as 1:2 epoxy: propylene oxide) into a reagent reservoir.
   c. Empty capsules of prior reagent or other fluid, if necessary.
   d. Depress pipettor plunger to the first stop.
   e. Insert the capsules into the resin-intermediate solvent and very slowly aspirate into the mPrep/s capsules.
      - ***Caution:*** *These reagents require very slow aspiration!*
   f. Wait the appropriate amount of time to ensure reaction, keeping the pipettor upright.
      - *It is recommended to use a lab stand to hold the pipettor and to rest capsule tips in the mPrep/bench rack (or on Parafilm or foil) to reduce any clean-up.*
      - *If desired, when incubation begins any fresh reagent remaining in the reservoir may be returned to storage for later use.*
   g. Slowly dispense used resin-intermediate solvent from the capsules to waste.

h. Repeat with more concentrated resin solutions (such as 1:1 epoxy: propylene oxide, perhaps followed by 100% epoxy without accelerator).

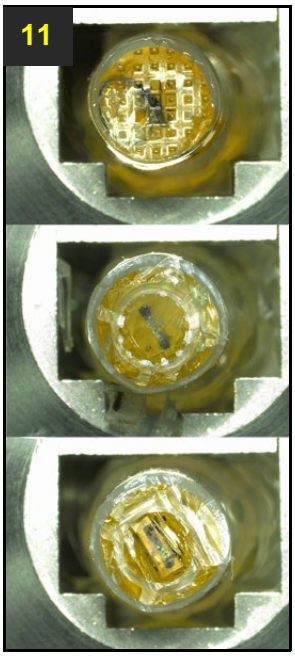
9. Embedding
   a. Pour sufficient 100% resin (such as epoxy) with accelerant into a reagent reservoir.
   b. Empty capsules of prior reagent or other fluid, if necessary.
   c. Depress pipettor plunger to the first stop.
   d. Insert the capsules into the 100% resin and very slowly aspirate into the mPrep/s capsules.
      - ***Caution:*** *These reagents require very slow aspiration!*
   e. Fully insert the resin-filled mPrep/s capsules into the wells of an mPrep/bench holder. Then press the eject button on the pipettor to eject the capsules into the mPrep/bench.
   f. Carefully disconnect the mPrep/f couplers (or empty mPrep/s capsules) from the resin-filled capsules, making sure the resin-filled mPrep/s capsules remain in the mPrep/bench holder.
   g. To form larger blocks most users will wish to "top off" the partially resin-filled mPrep/s capsules. To do this, use a disposable tip on the pipettor (or an ordinary pipette) to completely fill the resin-filled mPrep/s capsules with 100% resin with accelerant.
   h. If desired, insert additional provided labels into the resin for permanent embedding.
      - *The two capsules at the right of fig. 10 show labels inserted in the resin.*
   i. Place the mPrep/bench containing the specimen and resin-filled capsules into the oven for curing.

mPrep/bench with resin-filled capsules

Sectioning specimens in mPrep/s capsules

Sectioning embedded specimens in mPrep/s capsules is efficient and easy. The block does not need to be removed from the capsule in most cases, saving you time and effort.

1. Remove polymerized resin-filled mPrep/s capsules from the mPrep/bench. Snap off any excess epoxy which may have spilled.
2. Directly clamp the mPrep/s capsule into the microtome chuck.
   - *The mPrep/s capsule is sized to fit into standard microtome chucks. Usually there is no need to remove the block from the capsule, because its walls are sufficiently thin to allow the block inside to be securely clamped.*
   - *If you wish, the block can be removed by cutting away the capsule.*
3. Trim away sufficient mPrep/s capsule to expose the end of the block. Then face the block as usual prior to sectioning (series of photos in fig. 11).
   - ***Note:*** *Very little trimming is necessary because, as shown in the photos, the specimen is very close to the end of the capsule.*
4. Store capsules in mPrep Capsule Grid Box supplied with mPrep/s capsules.



Fluid reagent processing for SEM

Follow the fixation and dehydration methods for TEM fluid processing, above, as appropriate for your specimen and chemical protocol.

For air-drying from an organic solvent (such as HMDS)

1. Aspirate the air-drying reagent into the mPrep/s capsules.
2. Eject capsules from pipettor into the mPrep/bench. The mPrep CPD immersion holder or suitable, chemically-resistant 96-well plate may also be used.
3. Carefully disconnect the mPrep/f couplers (or empty mPrep/s capsules) from the filled capsules.
4. Let air-dry.

For drying by the critical point method

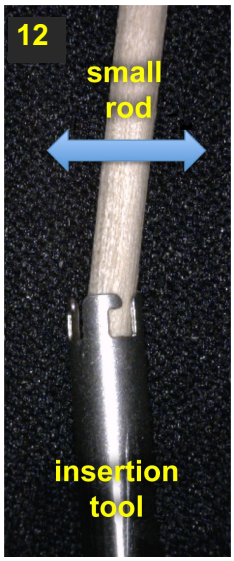
1. After dehydration in pure ethanol, eject the ethanol-filled capsules into an mPrep/bench or a 96-well plate that is partially filled with pure ethanol.
2. Carefully disconnect the mPrep/f couplers (or empty mPrep/s capsules) from the filled capsules while keeping filled mPrep/s capsules immersed in ethanol so that samples do not inadvertently air-dry.
3. Place the filled capsules into the mPrep CPD Immersion Holder and process by the critical point method. (See directions for mPrep CPD Immersion Holder.)

Mounting air- or CPD-dried SEM specimens

1. Using the insertion tool, remove the screen from the mPrep/s capsule.
2. If the specimen was not attached to the screen for orientation, remove SEM specimen from the capsule and mount in the usual manner.
3. If the specimen was oriented using the pinching method, it can remain in the screen for mounting:
   a. Remove the screen from the insertion tool with tweezers.
   b. Place it on the SEM stub and adhere with suitable adhesive.
   c. Because the screen is non-conductive polypropylene, use a conductive paste to provide a path to ground from the specimen to the SEM stub.

ADDITIONAL TECHNIQUES

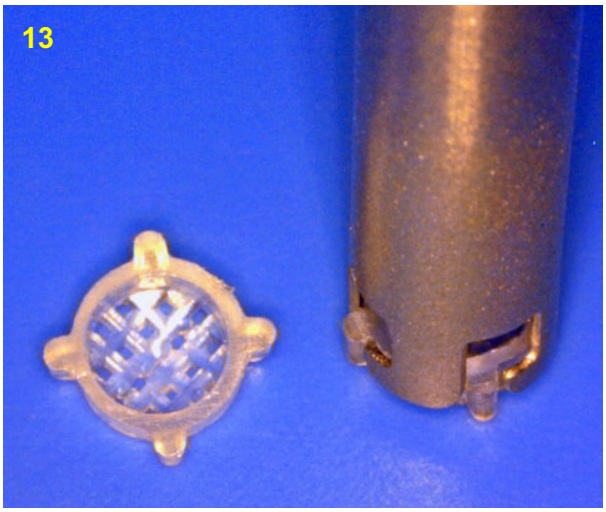
Trouble-shooting if insertion tool won't engage screen tabs



- Sometimes the insertion tool won't properly engage with screen tabs because its hooks are slightly bent inward.
- The solution:
  1. Place an applicator stick (or similar small rod) into the insertion tool as shown in fig. 12 and gently and very slightly bend the tabs out.
  2. Slide the insertion tool into an empty mPrep/s capsule and spin the tool relative to the empty capsule.
  3. The tabs will now conform to the inside dimension of the mPrep/s capsule and the tool should now properly engage and disengage with the screen.

Loading loose screens onto the insertion tool

- Extra screens are provided with mPrep/s capsules in case some are lost or require experimental modification.
- To load a loose screen onto a handheld insertion tool:
  1. Place a screen concave side facing up (fig. 13, left side).
  2. Align insertion tool over the screen with tabs aligned as shown in fig. 13 on the right side.
  3. Pick up the insertion tool and screen together and invert the assembly.
  4. As necessary, use fingers or other tool to rotate the screen clockwise to fully engage the insertion tool hooks onto the screen tab.
- To load a loose screen onto the workstation insertion tool:
  1. Using forceps, pick up a screen with the convex side up.
  2. Place it in the insertion tool being careful to align the screen tabs to slide into the insertion tool hooks.
  3. With forceps, rotate the screen tabs to engage with the insertion tool hooks.



Modifying screens

- Screens may be modified to hold particular types of specimens. For example, small holes of a desired shape may be cut in the screen using a scalpel, drill or punch to better clamp a particular specimen.

Orienting specimens using only the insertion tool

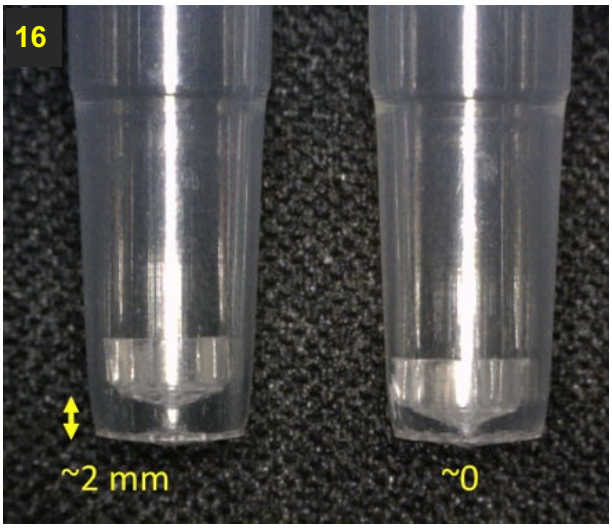
- Although the mPrep/s Workstation is recommended for orienting specimens, it is possible to accomplish two orientation methods with just the insertion tool, as follows:
    1. Hold the insertion tool vertically by hand, in a vise or in a custom jig. Position the insertion tool so that the screen, when loaded, will be at the top.
    2. Use either the “glue method” or “compression method” to achieve orientation, following the steps noted above in “Loading specimens into capsules — with orientation.”

Using the screen upside down for a concave surface

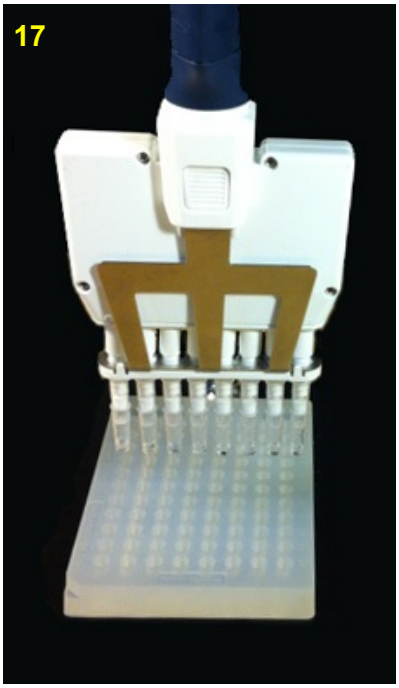
- The screen can be loaded into the insertion tool in the upside down orientation, so that the concave side faces the specimen.
- This configuration can be used to hold long specimens, as shown in fig. 15, as well as other types of specimens such as firm pellets.

Conserving expensive reagents

- Methods such as immuno-gold labeling of tissue require very expensive reagents. It is desirable in these situations to use as little reagent as possible.
- Processing small specimens in mPrep/s capsules can be accomplished with as little as 10 µl of antibody or other expensive reagents.
- The technique requires positioning the screen at its lowest possible point in the capsule.
    - *Normally there is a 1-2 mm space between the screen and the inner surface of the capsule since the insertion tool hooks prevent the screen from "bottoming out" in the capsule (fig. 16, left side).*
    - *This space can be reduced to near zero to hold very small specimens against the bottom of the capsule, thus reducing the amount of reagent required to cover the specimen.*
- The technique is as follows:
    1. Insert specimen and screen in the usual manner.
    2. Disengage insertion tool hooks from the screen tabs and partially remove the insertion tool.
    3. Slightly rotate the tool so that the hooks will not engage with the tabs.
    4. Use the insertion tool to press the screen further down into the capsule.
- Minor Cautions:
    - *Although seldom required, later removal of the screen is made more difficult by this procedure.*
    - *After processing with expensive reagents (e.g., antibodies or gold labels) it is recommended to reset the pipettor to a greater volume to ensure complete immersion and reactions with fixatives, solvents and other reagents.*

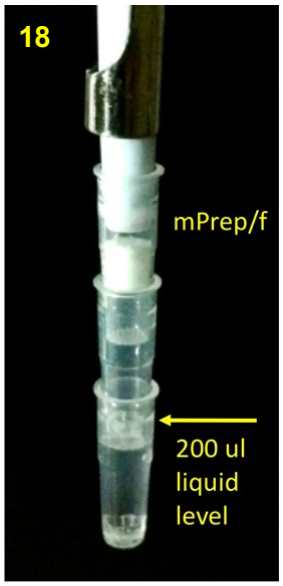


Removing reagent-filled capsules from the pipettor for microwave, incubator, oven or other long-term processing



- Reagent-filled mPrep/s capsules may be disconnected from the pipettor for microwave processing, long incubations or elevated-temperature incubations in a conventional oven or incubator.
- Filled capsules are placed into the mPrep/bench rack and, later, reattached to the pipettor using this technique:
  1. Fill the mPrep/s capsules in the usual manner.
  2. Using the pipettor, insert capsules into the mPrep/bench rack.
  3. Without touching the plunger, eject the capsules (including stacked capsules or mPrep/f couplers) from the pipettor into the mPrep/bench rack.
     - *The mPrep/bench provides a tight seal to retain fluid within each capsule. Other holders, such as conventional 96-well plates or vials, are acceptable but may allow some liquid to leak out.*
     - ***Warning:** To assure that capsules remain filled as they are ejected, it is important not to touch the plunger.*
  4. Reattach the capsules (or capsule stack) to the pipettor:
     (a) First, depress the plunger on the pipettor all the way to the second stop.
     (b) Insert the pipettor into the capsules, making sure they attach to the pipettor.
     (c) Slowly allow the plunger to move upward, aspirating into the capsule any reagent which may have leaked into the well.
        - *With the tight seal of the mPrep/bench rack, it may be necessary to rock the pipettor slightly to release the seal around the capsules and allow the liquid to be aspirated.*
     (d) Carefully lift the pipettor, making sure the capsules stay attached.

Stacking mPrep/s capsules



- In some cases, stacking mPrep/s capsules makes it possible to process two specimens on a single pipettor channel.
  - *Because the maximum capacity of an mPrep/s capsule is 150 µl, a standard 200 µl pipettor cannot entirely fill two capsules.*
  - *The upper mPrep/s capsule will only be partially filled to about the 40 µl level, as shown in fig. 18.*
- The stacking technique works with single- and multi-channel pipettors.

Immersion processing

- Another capability of mPrep/s capsules is that they may be used by fully immersing in liquids or gasses. This includes immersion in critical point drying apparatus, vapor staining, immersion in cryogens and other processes.
- Immersion holders for mPrep capsules are available for popular critical point dryers and other instruments. A holder for a critical point dryer is illustrated in fig. 19. Contact Microscopy Innovations for more information.

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Additional protocols

- Contact Microscopy Innovations or visit microscopyinnovations.com to obtain more detailed protocols for processing with mPrep/s capsules.
- If you need advice on modifying or developing a protocol, Microscopy Innovations is eager to assist you. Please contact us.
- Your ideas for new protocols or applications are also welcome. We are eager to learn from our valued customers. We encourage you to contact us.

NOTICES

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