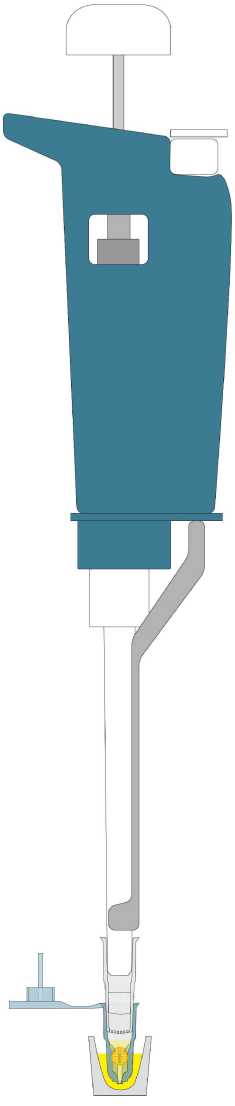
Processing TEM Grids with mPrep/g™ Capsules

mPrep/g™ capsules provide a processing, storage and archival system for TEM grids that:

- Protects TEM grids from loss, misidentification, and damage.
- Reduces the manual handling of grids.
- Reduces the amount of lab supplies utilized to process grids.
- Reduces reagent consumption and waste by over 75%.
- Pays for itself in reduced reagent use and by never losing an important grid.



Summary of mPrep/g™ device function:

- The mPrep/g capsule holds TEM grids within a pipette-like tube. The capsule attaches to common lab pipettors. Stains and other reagents are delivered to the grid by pipette action, as shown in drawing on the right.
- The mPrep/g capsule function reduces grid handling and provides a permanent storage solution. Barcode and/or alphanumeric labels provide a unique identity to each capsule, as shown in photo above.
- Grids are held with Grid-Tite™ technology, so they are firmly retained yet readily removed. Designed with advanced computational fluid dynamics, Grid-Tite provides excellent fluid access to enable complete and uniform staining.
- Grids stay in the capsule for staining. Since grid handling during staining is eliminated, they can't be damaged, lost or mixed up.
- Once a grid is prepared it never needs to leave its protective and labeled capsule, except when removed for electron microscopy.
- Grids in mPrep/g capsules are stored in convenient, project-size mPrep Capsule Grid Boxes, which are provided along with labeling materials. There is no need to transfer grids to a conventional grid box that requires a separate document to note grid identity.

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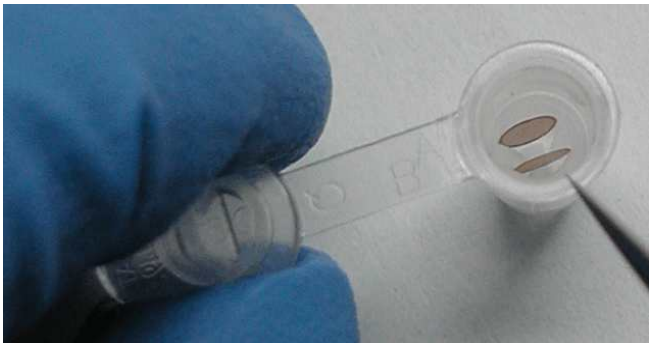
INSTRUCTIONS

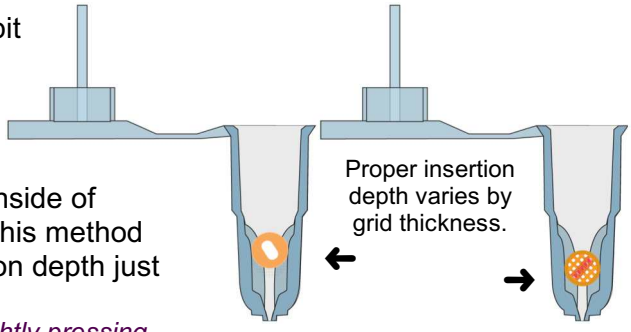
Equipment Needed

- **mPrep/g capsules**
- **mPrep/f couplers** — Use one coupler per pipettor channel
- **Labels** — a set of four identifying labels is supplied with each capsule.
- **TEM grids** — the capsules will retain virtually every type of 3 mm grid.
- **Forceps** — for grid handling, curved forceps such as Dumont #7 are recommended.
- **P200 Pipetman®** or equivalent laboratory pipettor capable of delivering 35 - 200 µl
  - *mPrep/g capsules are designed to fit on most brands of single- or multi-channel laboratory pipettors. Instructions are provided here for a single-channel Gilson P200 Pipetman. There may be slight differences if using other lab pipettor devices.*
- **Optional Equipment:**
  - pipette tips — to extend reach into solution vials
  - reagent reservoirs — useful for supplying reagents to multi-channel pipettors.

Preparation and insertion of grids into the mPrep/g capsules

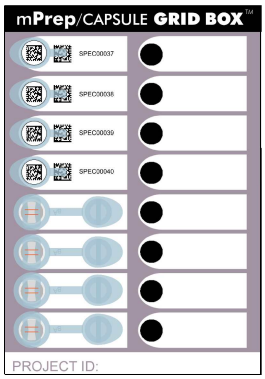
1. Prepare grids in your standard manner prior to insertion into mPrep/g capsules.
   - *Each mPrep/g capsule has slots for up to two grids, marked A and B on the hinge next to the capsule. See photo.*
2. Insert grid into the A or B slot, using one of two methods.
   Method 1: Hold capsule lid in left hand (right-handed instructions) with capsule held vertically. Tilt capsule slightly from the vertical — away from yourself to insert a grid into the far (A) slot or toward yourself for the near (B) slot. Then, hold grid against the inside of the capsule on the far or near side and release it. Gravity will usually slide grid into the slot. (You may have to adjust the grid angle a bit or gently tap the capsule bottom on the table.) Finish the process by gently pushing the grid about 1 mm more into the slot with forceps.





   Method 2: Hold grid with forceps against inside of capsule and simply guide it into the slot. This method takes practice to get the angle and insertion depth just right, but it is fast.
   - *Grids are gently retained as a result of slightly pressing against the slots. It is OK for the grids to be slightly curved.*
   - *Grid-Tite slots taper to accommodate grids of different thickness. Diagram at right shows that a thick (~50 µm) slot grid is inserted less than a thin (~20 µm) mesh grid. The photo below it depicts a thin mesh grid.*
   - ***Important! Do NOT insert grids to the bottom of the slots!** The slots extend to the bottom of the capsule to ensure fluid flow in and out of the capsule, not to indicate insertion depth.*

3. Insert second grid into capsule (optional).
4. Test for proper insertion — a properly inserted grid will not fall out even when the mPrep/g capsule is dropped, or is turned upside down and gently tapped on a table.
   - *We recommend practicing once or twice and testing with each type of grid you will use, prior to handling important specimens.*
5. Use supplied labeling system to document specimen identification.
   - *Four pre-numbered labels are provided for each capsule: a circular bar code or alphanumeric label for the top and three rectangular labels, which can include both alphanumeric and bar code. Place labels on capsule sides, in the mPrep Capsule Grid Box, on lab records, storage containers, etc.*
   - *Custom labels may be requested from Microscopy Innovations.*
   - *Diagrams at right and photo on page 1 show proper position of labels on capsule to avoid reagent exposure and intrusion into area where capsules mate with other capsules or pipette tips.*
6. Close capsule lid, if desired, to further protect grids.
7. Place capsules in mPrep Capsule Grid Box, a convenient project-sized storage solution.
   - *Grids will remain in mPrep/g capsules for staining and, later, for archival storage.*
   - *Remove grids only to place in the electron microscope.*

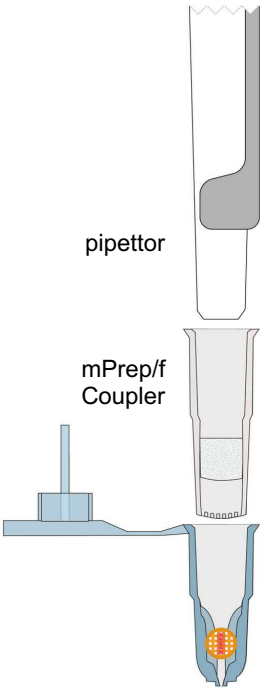


Staining grids using mPrep/g capsules

Any staining or immunolabeling method, as well as other fluid processing of grids, may be accomplished in mPrep/g capsules. Detailed protocols for uranyl acetate-lead citrate staining and immunolabeling are available from Microscopy Innovations at microscopyinnovations.com or by request.

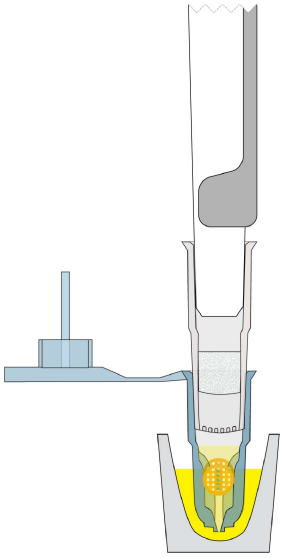
The general procedure is as follows:

1. <u>Prepare Pipettor & Attach Capsules</u>:
   a. Set volume of a P200 Pipetman (or equivalent pipettor) to 35 µl.
   b. Connect an mPrep/f Coupler™ to the Pipetman.
      - *This is recommended to prevent accidentally aspirating stains into the pipettor. The mPrep/f Coupler (shown) has a filter, which provides additional protection to the pipettor.*
   c. Connect the mPrep/g capsule containing your grids onto the coupler or empty capsule.
      - *Diagram shows the mPrep/f coupler and mPrep/g capsule aligned but not connected together.*



2. <u>One-time Setup</u> (confirms proper volume necessary to cover the grid):
   a. Depress the Pipetman plunger to the first stop position.
   b. Insert the tip of the mPrep/g capsule into water.
   c. Allow the plunger to <u>slowly</u> move up to aspirate water into the capsule.
   d. View the water level in the capsule to determine that the grid is covered.

e. Dispense the water from the mPrep/g capsule by depressing plunger to the first stop, pausing for ~1 second, and then depressing plunger to the second stop to purge any remaining water.
f. Adjust the volume as needed and repeat steps 2a-e.
   - *Once volume is determined, it does not need to be readjusted.*
   - *The volume required will increase if multiple mPrep/g capsules are stacked or if pipette tips are used to extend reach, as discussed below in Additional Techniques.*

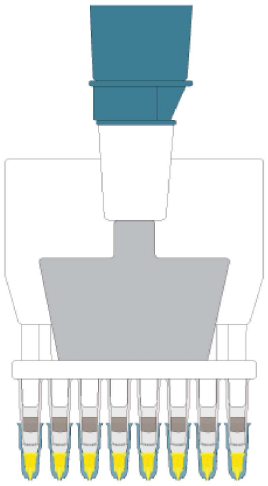


3. Staining:
   a. Depress the Pipetman plunger to the first stop position.
   b. Insert mPrep/g capsule into the first staining solution.
   c. Allow the plunger to slowly move up to aspirate staining solution into the capsule.
      - *Diagram shows the mPrep/g capsule being filled with stain.*
   d. Wait the required amount of time to ensure reaction, keeping the Pipetman in a vertical position.
      - *A lab stand can be used to hold the Pipetman vertically.*
      - *Keep the mPrep/g capsule resting in the stain reagent vessel. Alternatively, you may place the capsule tip on Parafilm or insert into an mPrep/bench™ silicone rack to provide a tight seal around capsules.*
   e. Dispense the staining solution by depressing the plunger to the first stop.
   f. Rinse thoroughly by inserting mPrep/g capsule into rinse solution. Rapidly aspirate and dispense 10 times. Repeat the 10 rapid rinses with fresh rinse solution, and repeat again for a total of 30 rapid rinses.
      - *Multiple rinses are required since mPrep/g capsule volume is intentionally small to minimize reagent consumption and facilitate grid insertion. They can be accomplished easily and rapidly.*
   g. Insert capsule into next stain, aspirate, and hold for desired time as described above for first stain. Then dispense stain to waste.
   h. Rinse thoroughly, as described above.
   i. After the final stain and rinse, depress the plunger to the first stop, pausing for ~1 second. Then depress plunger to the second stop to purge any remaining solution while blotting the ends of the capsules on absorbent paper.

4. Removal and Storage:
   a. Remove the grid-containing mPrep/g capsules from the pipettor after purging (step 3i).
      - *Grids may retain residual rinse water. Ordinarily this is not a problem with clean rinse water, as it will eventually evaporate leaving no deposits on the grid. If faster drying is important, insert absorbent paper into the capsule to touch the point where the grid's edge contacts the capsule. This will wick out the water.*
   b. Place open mPrep/g capsules into the mPrep Capsule Grid Box so that grids can fully air dry. Then close the top of the capsule for storage until removal for imaging.
      - *If you are in a hurry to perform imaging and grids are still damp, do what you always used to do — remove the grid from the capsule with a forceps, blot the grid, then place it in the microscope stage.*
   c. Use forceps to remove the grid from the mPrep/g capsule for imaging. When imaging is complete, return the grid to the mPrep/g capsule for archival storage in the mPrep Capsule Grid Box.

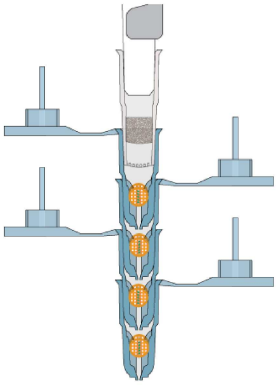
ADDITIONAL TECHNIQUES

Staining multiple grids using a multi-channel pipettor



- An efficient way to stain multiple grids is with the use of an 8- or 12-channel pipettor. It allows high-throughput manual processing of dozens of grids at a time. Each grid receives exactly the treatment — creating very consistent staining results.
- It is even possible to follow different staining or labeling protocols on each channel. This is conveniently accomplished by laying out reagents in 96-well plates.
  - *This technique is described within the "Immunolabeling with mPrep™ Capsules Protocol," available from Microscopy Innovations at microscopyinnovations.com or by request.*

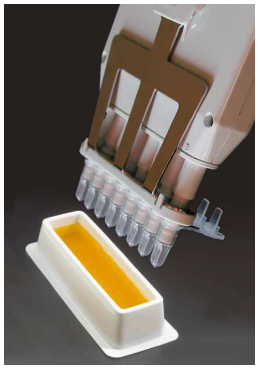
Staining multiple grids by stacking mPrep/g capsules



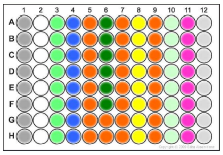
- Multiple grids may also be stained simultaneously by stacking mPrep/g capsules (drawing at right).
  - *Be sure to increase the Pipetman volume to cover all grids using One-time Setup, as described above.*
- This technique works with single- and multi-channel pipettors.
  - *4-5 capsules may be stacked per channel, depending on the pipettor's volume.*
- The following chart shows the increased grid processing capacity that stacking allows for various sized pipettors.

		Grid capacity, based on pipettor type		
		Single channel	**8-channel**	**12-channel**
	1 capsule	2	16	24
Stacking	**2 capsules**	4	32	48
	3 capsules	6	48	72
	4 capsules	8	64	96

Using reagent reservoirs with multi-channel pipettors



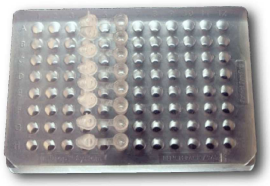
- Reagents can be conveniently and efficiently supplied to multi-channel pipettors from reagent reservoirs or 96-well plates. These allow simultaneous filling of all 8 or 12 channels with little waste. Microscopy Innovations offers reservoirs and plates tested for system compatibility.
- Reagent reservoirs with 15-100 ml capacity are recommended for grid staining with 8- or 12-channel pipettors.
  - *Air- or light-sensitive and contamination-prone stains (such as uranyl acetate or lead citrate) should be dispensed into reservoirs immediately prior to use and/or kept covered.*
  - *Larger volume reservoirs are appropriate for rinses or less expensive reagents.*
- 96-well plates provide low-volume, individual wells. They are recommended for more expensive reagents, such as those used in immuno-gold protocols.



Extending reach into reagent containers

- Standard pipette tips may be attached to mPrep/g capsules to extend their reach into a solution vial. This also reduces the potential for contamination of a stain solution. Capsules mate with Gilson size D200 and DL10 (or equivalent) pipette tips. These extension tips may be removed and discarded after each reagent is aspirated and dispensed. The pipettor volume will need to be increased. (See One-time Setup, above.)

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



- Reagent-filled mPrep/g capsules may be removed 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 or other suitable holder and, later, reattached to the pipettor using this technique:
  1. Remove any pipette tips attached to the reagent-filled mPrep/g capsules.
  2. Without touching the plunger, eject the capsules (or stack of capsules) from the pipettor into the mPrep/bench™ rack or other holder.
     - *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.*
  3. Reattach the capsules (or stack of capsules) to the pipettor:
     (a) First, depress the plunger on the pipettor to the first 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 staining solution 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.*

Immersion processing

- Another capability of mPrep/g 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.
- Prior to placing the mPrep/g capsule into the immersion holder, it is recommended to enlarge the hole in the bottom of the capsule to ensure adequate filling.
- Immersion holders for mPrep capsules are available for popular critical point dryers and other instruments. A holder for a critical point dryer is illustrated at right. Contact Microscopy Innovations for more information.

Additional protocols

- Contact Microscopy Innovations or visit microscopyinnovations.com to obtain more detailed protocols for staining and immunolabeling with mPrep/g capsules.
- If you need advice on developing a protocol, Microscopy Innovations is eager to assist you. Please contact us.

NOTICES

Product Warranty

Microscopy Innovations, LLC warrants the mPrep sample system against defects in materials and workmanship under normal use for a period of thirty (30) days from the date of retail purchase by the original end-user purchaser ("Warranty Period"). If a defect arises and a valid claim is received by Microscopy Innovations within the Warranty Period, the end-user purchaser's sole remedy, and Microscopy Innovations' sole obligation shall be, at Microscopy Innovations' discretion, either (1) replacement of the product, or (2) refund the purchase price of the product.

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