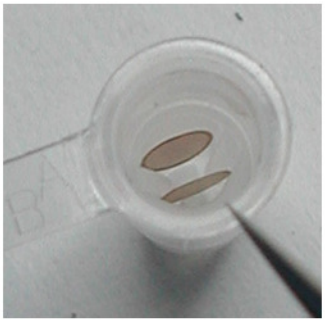
Staining Grids with mPrep/g Capsules

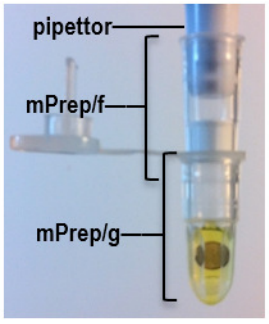
mPrep™ System

Protocol

This document illustrates how to use the mPrep System with the most common biological section grid staining method (uranyl acetate and Reynolds' lead citrate). This method allows simultaneous staining of multiple grids for efficiency and consistent results.



Inserting grids into capsule



Staining grids in capsule

Storing grids in capsule grid box

Preparing for TEM insertion

Choose between two protocols by the number of grids you need to stain, using 1–2 grids per mPrep/g capsule:

Run	Use	Stain	Maximum Capacity per Pipettor
Protocol I	single channel pipettor	1–2 grids in 1 capsule	up to 8 grids in one 4-capsule stack
Protocol II	8-channel pipettor	8–16 grids in 8 capsules	up to 64 grids in eight 4-capsule stacks
	12-channel pipettor	12–24 grids in 12 capsules	up to 96 grids in twelve 4-capsule stacks

Equipment and Supplies

Item	Additional information
mPrep/g capsules	Use mPrep/g capsules for processing TEM grids.
mPrep/f filter couplers	Use 1 coupler per pipettor channel to minimize potential contamination with stains or reagents and enable firm attachment of capsules to pipettors (e.g., Gilson Pipetman Neo® device).
grids	Insert 1–2 grids with sections into each mPrep/g capsule before beginning protocol.
pipettor	Use a single- or multichannel lab pipettor with 200 µl capacity that fits mPrep Capsules (e.g., single or multichannel Gilson Pipetman Neo® device).
staining reagents	Prepare uranyl acetate and Reynolds' lead citrate reagents. Because stain formulae and timing vary with the sections being stained, not all details are provided. Adapt this protocol using your current stains and timing, and optimize as appropriate for your conditions.
water for rinses	Use ultrapure, distilled, or deionized water. Some users prefer an alternative rinse solution.
containers for reagents, rinses, and waste	Use beakers, vials or multi-well vessels when using a single channel pipettor. Use trough-style reagent reservoirs, 12-column plates and/or 96-well plates when using a multichannel pipettor.
Parafilm® or similar material	Use as a temporary seal during staining steps.
absorbent paper	Use filter paper, filter paper wedges, or lab wipes to remove liquids during drying steps.
aluminum foil	Use aluminum foil or other method to block light during uranyl acetate staining.
laboratory stand (optional)	Use to hold the pipettor vertically when resting on the Parafilm material during staining steps.

MICROSCOPY INNOVATIONS™ LLC
213 Air Park Rd Suite 101
Marshfield WI 54449-8626
888.302.3925 or 715.384.3292
info@microscopyinnovations.com

Protocol I

Stain up to 8 grids using a single channel pipettor

Before you begin: Assemble equipment and supplies as noted above and shown in Figure A. Insert grids with sections into mPrep/g capsules (1–2 grids per capsule). Prepare reagents (e.g., centrifuge or filter uranyl acetate and Reynolds' lead citrate stains), and dispense liquids into containers. Attach mPrep/g capsules onto pipettor:

a. Place an mPrep/f coupler onto pipettor.
b. Attach mPrep/g capsule or capsules with grids to coupler (Figure B).
c. *Optional:* Stack up to 4 grid-containing capsules on a single pipettor channel (Figure C).

2. Set pipettor volume by number of capsules to be stacked on the pipettor:

Number of capsules	1	2	3	4
Volume	35–40 µl	80 µl	120 µl	160 µl

3. Stain with uranyl acetate:

a. Aspirate prepared uranyl acetate into capsules (Figure B, C).
b. Place tip of mPrep/g capsule on Parafilm (Figure D).
c. Cover with foil to protect uranyl acetate stain from light.
d. Hold for desired staining time, typically 3–15 minutes.
e. Dispense into waste.

4. Rinse using a series of rapid exchanges:

a. Set up 5 plate wells or reservoirs filled with fresh rinse solution.
b. Aspirate and dispense rapidly 10 times in each well or reservoir. Proceed through all 5 wells/reservoirs for a total of 50 exchanges.

Note: You can easily do all 50 rinses in about 1 minute. Repeated rinses are required because the mPrep/g volume is small and rapid rinsing generates circulation in the capsule to remove excess stain. It is important to proceed to a fresh rinse reservoir after every 10 rinses.

5. Stain with Reynolds' lead citrate:

a. Aspirate prepared Reynolds' lead citrate into capsules.
b. Place tip of mPrep/g capsule on Parafilm.
c. Hold stain inside capsule, typically 3–10 minutes (Figure D).
d. Dispense into waste.

6. Rinse with another 50 rapid exchanges:

a. Repeat step 4, ending with a dispense step.
b. Pause for ~10 seconds to drain.
c. Depress the dispense button completely to purge water from pipettor.
d. Place capsule tips onto absorbent paper to draw water from capsule.
e. *Optional:* Repeat purge step 6c to remove additional water.

Figure A: Preparation of equipment and supplies for Protocol I. Use a lab stand and clamp to hold the pipettor upright.

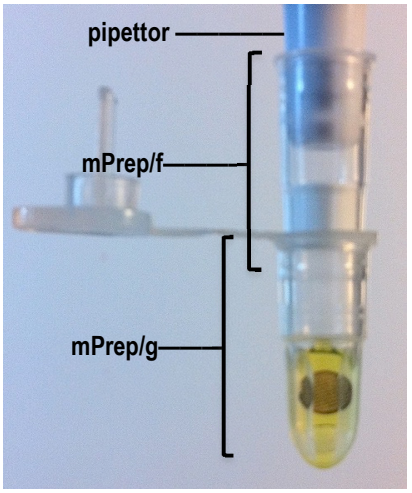


Figure B: Attaching an mPrep/g capsule to an mPrep/f coupler and pipettor.

Figure C: Stacking 4 mPrep/g capsules on a single-channel pipettor. Staining with uranyl acetate.

7. Dry and store:
    a. Remove mPrep/g capsules from pipettor and couplers, separating stacked capsules, if applicable.
    b. Blot off any water on capsule bottoms using absorbent paper.
    c. Place uncapped capsules in mPrep Capsule grid box.
    d. *Optional:* Insert absorbent paper (e.g., filter paper wedge) into the capsule to wick up water droplets where the grid edges meet the inside capsule sidewall.
    e. Air dry capsules in grid boxes open until fully dry.
    f. *Optional:* Accelerate drying by placing box on slide warmer.
    g. Close capsules and grid box for archival storage.

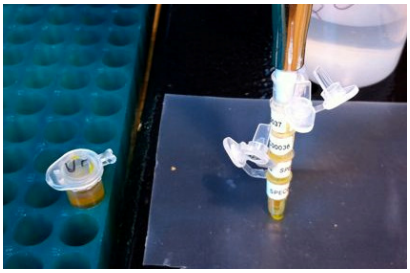


Figure D: Placing the tip of an mPrep/g capsule onto Parafilm.

Figure E: Preparation of equipment and supplies for Protocol II. Use a lab stand and clamp to hold the pipettor upright. 8-channel pipettor shown.

Protocol II

Stain 8 to 96 grids with a multichannel pipettor

Before you begin: Assemble equipment and supplies as noted previously and shown in Figure E. Insert grids with sections into mPrep/g capsules (1–2 grids per capsule), Prepare reagents (e.g., centrifuge or filter uranyl acetate and Reynolds' lead citrate stains), and dispense liquids into reagent reservoirs.

1. Load mPrep/g capsules onto an 8- or 12-channel pipettor (Figure F):
    a. Place an mPrep/f coupler onto each pipettor channel.
    b. Attach mPrep/g capsules with grids onto mPrep/f couplers.
    c. Stack up to 4 mPrep/g capsules onto each pipettor channel to enable simultaneously staining of up to 96 grids (with a 12-channel pipettor).

2. Set pipettor volume by number of capsules to be stacked on each channel of the pipettor:

Number of capsules	1	2	3	4
Volume	35–40 µl	80 µl	120 µl	160 µl

3. Stain with Uranyl Acetate:
    a. Immediately prior to filling capsules, pour or pipette prepared uranyl acetate stain into reagent reservoir (Figure G) with at least ~2 ml to cover the bottom of the reservoir.
    b. Aspirate uranyl acetate into all capsules from reservoir.
    c. Place tip of mPrep/g capsule on Parafilm (Figure H).
    d. Cover with foil to protect uranyl acetate stain from light.
    e. Hold stain inside capsule, typically 3–15 minutes.
    f. *Optional:* Pour unused stain into reagent vial for later use.
    g. Dispense into waste.

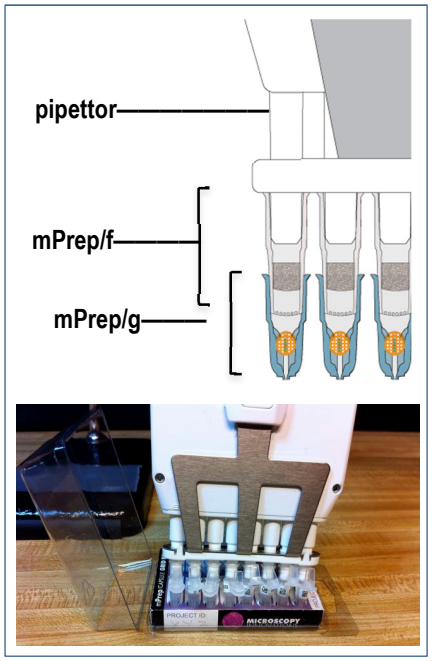


Figure F: Attaching an mPrep/g capsules to mPrep/f couplers and multichannel pipettor (top) and performing this step directly from an mPrep capsule grid box (bottom).

4. Rinse using a series of rapid exchanges:
   a. Set up 5 plate wells or reservoirs filled with fresh rinse solution.
   b. Aspirate and dispense rapidly 10 times in each well or reservoir. Proceed through all 5 wells/reservoirs for a total of 50 exchanges.

   Note: You can easily do all 50 rinses in about 1 minute. Repeated rinses are required because the mPrep/g volume is small and rapid rinsing generates circulation in the capsule to remove excess stain. It is important to proceed to a fresh rinse reservoir after every 10 rinses.

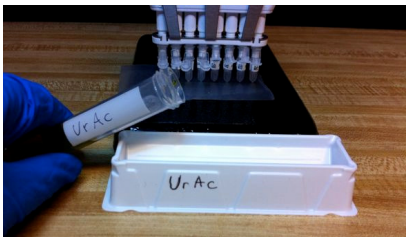


Figure G: Pouring stain into a reservoir.

5. Stain with Reynolds' lead citrate:
   a. Immediately prior to filling capsules, pour or pipette prepared Reynolds' lead citrate stain into reagent reservoir (Figure G) with at least ~2 ml to cover the bottom of the reservoir.
   b. Aspirate Reynolds' lead citrate into all capsules from reservoir.
   c. Place tip of mPrep/g capsule on Parafilm (Figure H).
   d. Hold stain inside capsule, typically 3–10 minutes.
   e. Dispense into waste.

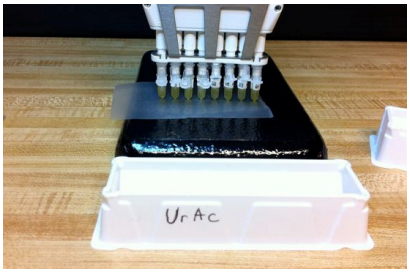


Figure H: Placing capsules on Parafilm during staining.

6. Rinse with another 50 rapid exchanges:
   a. Repeat step 4, ending with a dispense step.
   b. Pause for ~10 seconds to drain (Figure I).
   c. Depress the dispense button completely to purge water from pipettor.
   d. Place capsule tips onto absorbent paper to draw water from capsule (Figure J).
   e. *Optional:* Repeat purge step 6c to remove additional water.

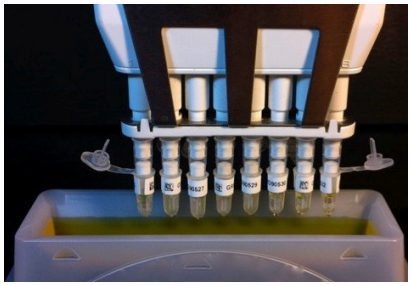


Figure I: Rinsing capsules in water from a reagent reservoir.

7. Dry and Store:
   a. Remove mPrep/g capsules from pipettor and couplers, separating stacked capsules, if applicable.
   b. Blot off any water on capsule bottoms using absorbent paper.
   c. Place uncapped capsules in mPrep Capsule grid box.
   d. *Optional:* Insert absorbent paper (e.g., filter paper wedge) into the capsule to wick up water droplets where the grid edges meet the inside capsule sidewall.
   e. Air dry capsules in grid boxes open until fully dry.
   f. *Optional:* Accelerate drying by placing box on slide warmer.
   g. Close capsules and grid box for archival storage.

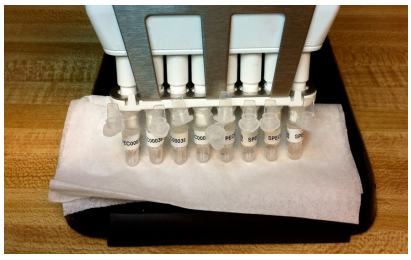


Figure J: Blotting excess fluid from mPrep/g capsules.

Related Documents

AN502 **Easy and Efficient Grid Staining of Biological Tissue for TEM**
Application Note features staining mammalian tissues with mPrep/g capsules.