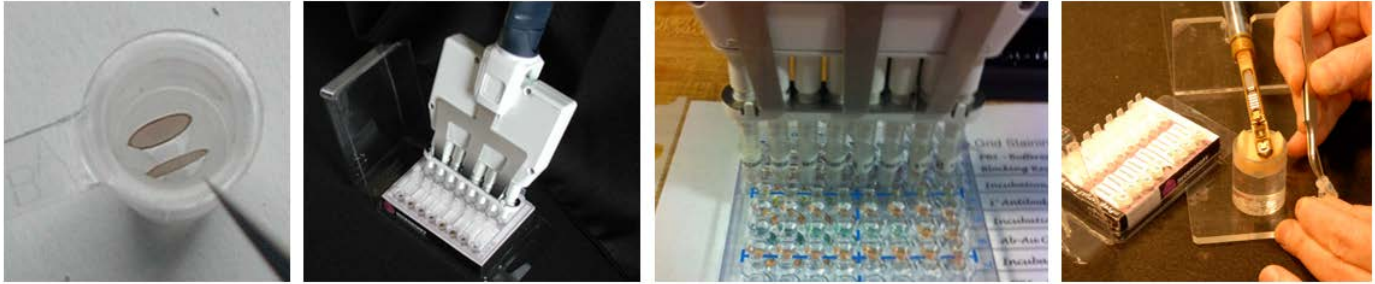


Immunolabeling protocols are easily adapted to mPrep/g processing. This document illustrates a typical protocol using a primary antibody and a secondary antibody conjugated to colloidal gold, followed by staining with uranyl acetate and lead citrate.



Inserting grids into capsule

Removing capsules from grid box

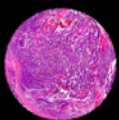
Immunolabeling using template guide

Preparing for TEM insertion

Immunolabeling is a powerful methodology for localizing structures in biological specimens. However, the customary method that transfers TEM grids among reagent droplets can require ~100 grid handling steps, carrying a high risk of damage and potential for grid mix-up. Conversion of the protocol to one using mPrep/g capsules ensures that grids are safely encapsulated, protected from damage, labeled for traceability, processed identically, and touched only twice—during insertion into the capsule at the microtome and again when placing in the microscope. Processing involves following a template to pipette reagents from a 96-well microplate.

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., a Gilson Pipetman Neo® device).
grids	Insert 1–2 grids with sections per mPrep/g capsule before beginning protocol.
pipettor	Use a multichannel lab pipettor with 200 µl capacity that fits mPrep Capsules (e.g., multichannel Gilson Pipetman Neo® device).
reagents*	Prepare reagents. For a typical immunolabeling protocol this may include phosphate buffered saline (PBS), blocking solution, incubation solution, primary antibody, colloidal gold conjugated to secondary antibody, uranyl acetate and Reynolds' lead citrate. Because formulae and timing will vary among investigations, only an example protocol is provided here. Adapt this to your labeling protocol and optimize as appropriate for your conditions. Note: If your protocol uses Triton®, Tween®, or other detergents, see Important Note When Using Detergents on page 3.
water for rinses	Use ultrapure, distilled, or deionized water.
reagent reservoirs	Use 2–4 trough-style reagent reservoirs for buffers and rinses.
96-well microplate	Use one low-adhesion type microplate with gently rounded or flat-bottom wells.
blank grid staining template	Use the printable template included in this document or downloaded here for protocol layout.
Parafilm® (or equivalent) or mPrep/bench 96	Use as a temporary seal during staining steps. The mPrep/bench is a silicone 96-well plate that provides a tight seal for capsule bottoms, enabling long incubations without fluid evaporation.
absorbent paper	Use filter paper, filter paper wedges, or lab wipes to remove liquids during drying steps.
aluminum foil	Use 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.
sheet protector	Use to protect the protocol template from spills.



Protocol

Protocol

Before you begin: Assemble equipment and supplies as noted above and shown in Figure A. Insert grids with sections into mPrep/g capsules (1 or 2 grids per capsule). Prepare reagents. Print and review the [example protocol template](#) (page 6) and [blank protocol template](#) (page 7).

1. Use the blank template to set up your protocol template, referencing the example protocol template:

Note: In the standard orientation (Figure B, portrait layout), one template can be used for up to 12 steps with an 8-channel pipettor. By rotating the template 90 degrees (landscape orientation), one template can be used for up to 8 steps with a 12-channel pipettor. Additional steps, if needed, can be mapped out on a second blank template.

- a. Start at the top of the template and write each protocol step on the blank horizontal lines aligned to the “row” wells in the microplate.

Tip: Reagents that require small volumes or titrations will be transferred into microplates. Inexpensive reagents and those that require large volumes, such as buffers and water, can instead be placed into reagent reservoirs. Be sure to note the reagent along with any dilutions, titrations and controls (see example template on page 6).

- b. Insert completed template into a clear sheet protector.
- c. Place a 96-well microplate over the template and secure with tape to maintain alignment of wells with template (Figure B).

2. Transfer reagents into microplate wells and reservoirs:

Important Note: The reagent volume in each microplate well must be sufficient to fill all mPrep/g capsules per pipettor channel. Typically this will be 1 or 2 mPrep/g capsules, but up to 4 mPrep/g capsules can be attached or stacked onto each pipettor channel to label as many as 8 grids per channel. To manage pipetting, the recommended microplate well volume in step 2a includes an excess of ~10 µl.

- a. Choose the microplate well volume for the reagents by the number of capsules to be stacked on each pipettor channel:

Number of capsules per pipettor channel	1	2	3	4
Microplate well volume	≥ 45 µl	≥ 90 µl	≥ 130 µl	≥ 170 µl

- b. Fill the wells according to the template created in Step 1.

Note: When filling microplate wells, be sure to fill wells across rows with the proper titrations and controls as per protocol. Rows that have the same reagent can be conveniently filled using a multichannel pipettor fitted with pipette tips; rows that contain different reagents or titrations must be filled individually with a single-channel or multichannel pipettor.

Important: To minimize air exposure, uranyl acetate and lead citrate stains should be placed into wells immediately before use.



Figure A: Preparation of equipment and supplies. Use a lab stand and clamp to hold the pipettor upright, the protocol template, and reservoir troughs.



Figure B: Microplate containing reagents placed on protocol template in portrait orientation to use with 8-channel pipettor.

3. Load mPrep/g capsules onto a multichannel pipettor (Figure C):

- a. Place an mPrep/f coupler onto each pipettor channel.

Important Note When Using Detergents: If your protocol uses Triton, Tween, or other detergents, bubbles may form during pipetting because the small holes at the end of mPrep/f couplers can act like an aerator. To prevent such bubbling, we recommend modifying the mPrep/f couplers by simply cutting off the bottom of each mPrep/f coupler using a razor or other sharp blade (Figure D).

- b. Attach mPrep/g capsules with grids onto mPrep/f couplers.
- c. *Optional:* 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).

Important: To ensure consistent filling with reagents, make sure that the mPrep/g capsules and mPrep/f couplers are securely attached.

4. Set pipettor and rinse the capsules with PBS:

- a. Set pipettor volume by number of capsules to be stacked on each pipettor channel:

Number of capsules per pipettor channel	1	2	3	4
Pipettor volume	35–40 μ l	80 μ l	120 μ l	160 μ l

- b. Insert capsules to bottom of reagent reservoir containing PBS.
- c. Depress the plunger of the pipettor to its first stop, and release it slowly to aspirate PBS into capsules, ensuring that all grids are immersed.

Important: If all grids are not immersed with the volumes shown in step 4a, depress the plunger of the pipettor to the second stop to dispense the PBS to waste, increase the volume setting on pipettor, and try again. If you see different fluid levels among capsules, this means a coupler is not tightly fitted to the pipettor barrel or a capsule is not tightly fitted to a coupler. Check the tightness of the fittings, and try again. Repeat until the fluid levels among all stacked capsules are consistent.

- d. Dispense PBS from capsules to waste.

5. Treat with first reagent.

Note: In the example the reagent in the first microplate row is the blocking reagent; reagents in your protocol may differ.

- a. Position the pipettor over the first microplate row and insert capsules to bottom of wells.
- b. Aspirate first reagent (blocking reagent in the example) into capsule (Figure E).
- c. Rest tip of mPrep/g capsules in first row of 96-well plate using a lab stand or other method to keep pipettor upright.
- d. Wait for time indicated in protocol.

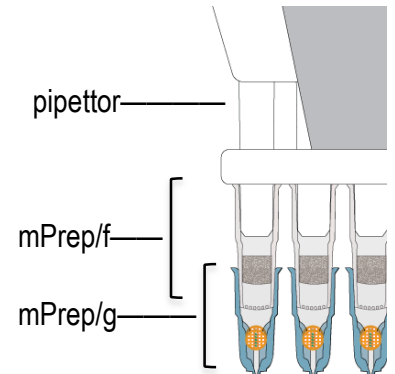


Figure C: Attaching an mPrep/g capsules to mPrep/f couplers and multichannel pipettor

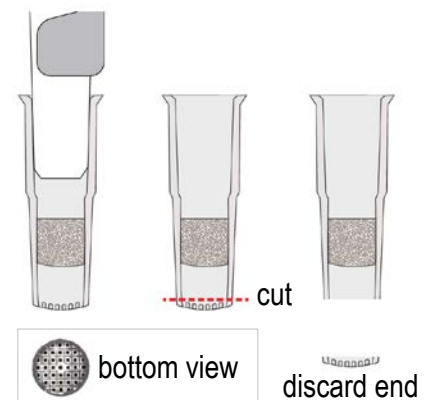


Figure D: Cutting off the end of the mPrep/f couplers minimizes bubbling when pipetting detergent-containing reagents.

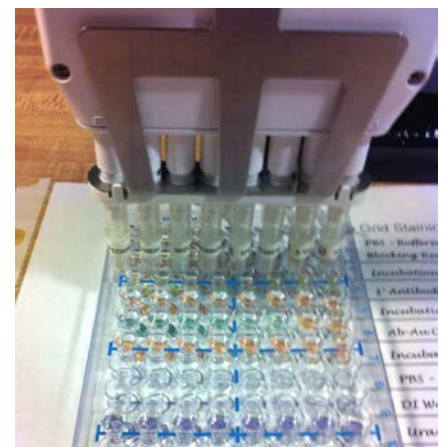


Figure E: Aspirating blocking reagent into capsules with 8-channel pipettor.

- e. Dispense first reagent back into well.
6. Treat with second and subsequent reagents.
 - a. Follow steps 5a–5d pipetting the second reagent (incubation reagent in the example) from the second microplate row.
 - b. Repeat steps 5a–5d, pipetting each immunolabeling reagent (primary antibody with controls or titers, incubation reagent, antibody-gold conjugate with controls or titers) in sequence from its corresponding row (3, 4, 5, or 6) in the microplate followed by PBS from its reagent reservoirs identified for row 7.

Important: For any incubation times 1 hour or longer, the mPrep/g capsule bottoms should be sealed to prevent excessive evaporation by inserting capsule bottoms into an mPrep/Bench silicone 96-well plate (not shown) or partially sealed by resting on Parafilm.

7. Rinse rapidly with water, using 5–10 exchanges:
 - a. Place mPrep/g capsule tips into a reagent reservoir filled with rinse water (example row 8).
 - b. Rapidly aspirate and dispense from this single reservoir to a total of 5–10 cycles.
8. Stain with Uranyl Acetate:
 - a. Immediately before staining, pipette freshly prepared (centrifuged or micro-filtered) uranyl acetate into microplate wells as shown on template. Alternatively, pour (Figure F) or pipette uranyl acetate stain into reagent reservoir with at least ~2 ml to cover the bottom of the reservoir.
 - b. Aspirate uranyl acetate into all capsules from microplate or reservoir.
 - c. Place tip of mPrep/g capsule on Parafilm (Figure G).
 - d. Cover with foil to protect uranyl acetate stain from light (not shown).
 - e. Hold stain inside capsule, typically 3–15 minutes.
 - f. *Optional:* Pour unused stain into reagent vial for later use.
 - g. Dispense into waste.

9. Rinse rapidly with water, using 24 exchanges (Figure H):
 - a. Place mPrep/g capsule tips into a reagent reservoir filled with fresh rinse water.
 - b. Rapidly aspirate and dispense from this single reservoir to a total of 8 cycles.
 - c. Refresh the rinse water.
 - d. Repeat steps 9b-9c for 8 cycles to a total of 16 cycles.
 - e. Repeat steps 9b-9c for 8 cycles to a total of 24 cycles.



Figure F: Pouring a reagent (in this case uranyl acetate stain) into a reservoir.



Figure G: Placing capsules on Parafilm during staining.

Protocol

Note: You can do all 24 rinses in ~1 minute—each aspirate and dispense cycle should only take 1–2 seconds. Repeated rinses are required because the mPrep/g volume is small and rapid rinsing generates circulation in the capsule to remove excess stain.

10. Stain with Reynolds' lead citrate:

- Immediately before staining, pipette freshly prepared (centrifuged or micro-filtered) Reynolds' lead citrate into microplate wells as shown on template. Alternatively, pour or pipette Reynolds' lead citrate stain into reagent reservoir with at least ~2 ml to cover the bottom of the reservoir.
- Aspirate Reynolds' lead citrate into all capsules from reservoir.
- Place tip of mPrep/g capsule on Parafilm.
- Hold stain inside capsule, typically 3–10 minutes.
- Dispense into waste.

11. Rinse rapidly with water, using 24 exchanges:

- Repeat step 9, ending with a dispense step.
- Pause for ~10 seconds to drain.
- Depress the dispense button completely to purge water from pipettor.
- Place capsule tips onto absorbent paper to draw water from capsule (Figure I).
- Optional: Repeat purge step 11c to remove additional water.

12. Dry and Store:

- Remove mPrep/g capsules from pipettor and couplers, separating stacked capsules, if applicable.
- Blot off any water on capsule bottoms using absorbent paper.
- Place uncapped capsules in mPrep Capsule grid box.
- 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.
- Air dry capsules in grid boxes open until fully dry.
- Optional:* Accelerate drying by placing box on slide warmer.
- Close capsules and grid box for archival storage.

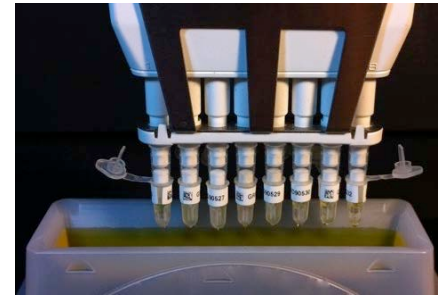


Figure H: Rinsing capsules in water from a reagent reservoir.



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

Related Documents

PROTOCOL Staining Grids with mPrep/g Capsules

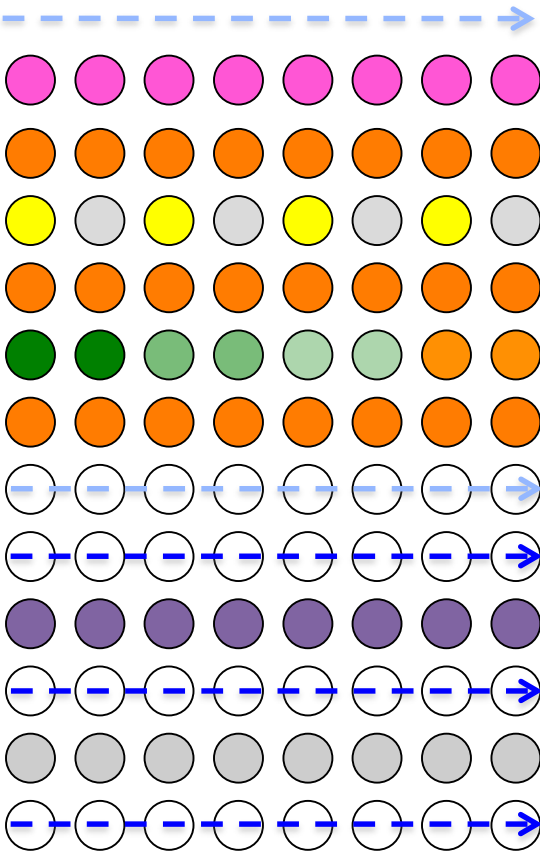
Protocol features uranyl acetate and lead citrate staining of grids with biological sections in mPrep/g capsules.

Available for download from www.microscopyinnovations.com

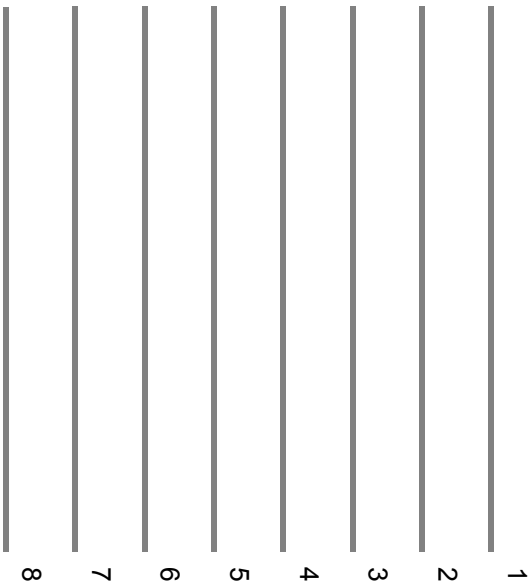
Protocol

Example Protocol Template

mPrep System 96-well-plate Grid Staining Template



PBS – Buffered Saline – in Reservoir	
Blocking Reagent (10 min)	1
Incubation Reagent (60 min)	2
1° Antibody and controls (60 min)	3
Incubation Reagent (10 min)	4
Ab-Au Conjugate Titrations (60 min)	5
Incubation Reagent (10 min)	6
PBS – Buffered Saline – in Reservoir (2 × 5 min rinses)	7
DI Water Wash – in Reservoir (5–10 rinses)	8
Uranyl Acetate Stain (10 min, covered)	9
DI Water Wash – in Reservoir (3 exchanges × 8 cycles = 24 rapid rinses)	10
Reynolds' Lead Citrate Stain (10 min)	11
DI Water Wash – in Reservoir (3 exchanges × 8 cycles = 24 rapid rinses)	12



Protocol Notes

Arrows indicate use of reservoir for reagent

1° Antibody and controls
 (1° Antibody = primary antibody)
 Yellow = primary antibody
 Grey = control with no antibody

Ab-Au Conjugate Titrations
 (Ab-Au = antibody-gold)
 Dark to Light Green = High to low titrations
 Orange = control with no Ab-Au

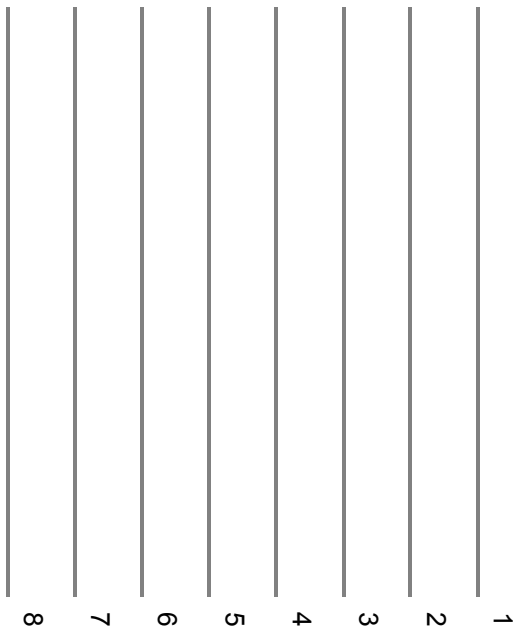
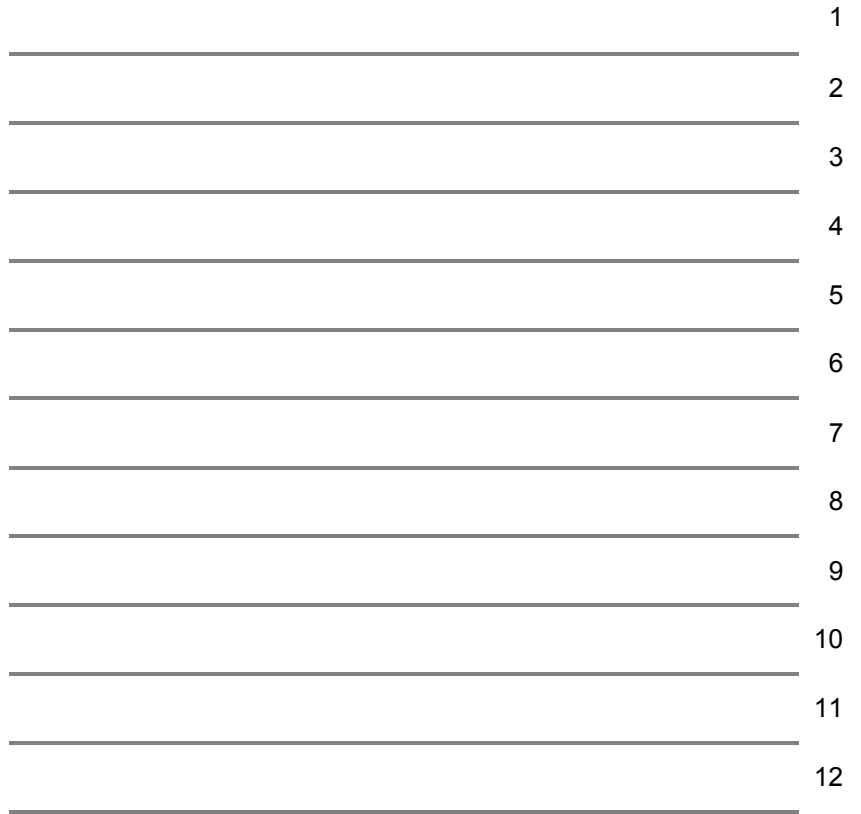
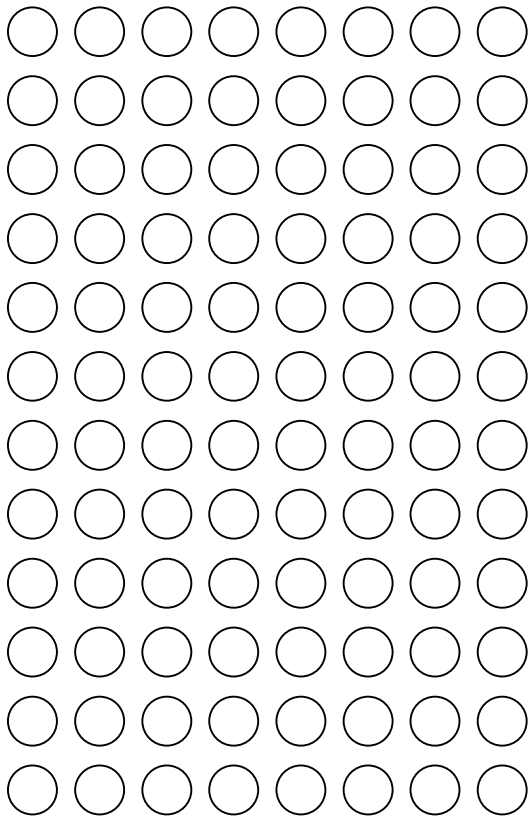
To maintain proper 96-well spacing, be sure to print this document without resizing.

Immunolabeling Grids with mPrep/g Capsules Protocol rev2

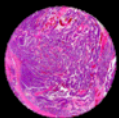
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Protocol Template

mPrep System 96-well-plate Grid Staining Template



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MICROSCOPY
INNOVATIONS LLC

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