Easy and Efficient Grid Staining of Biological Tissue for TEM

mPrep™ System

Applications Note #502

Introduction

Staining biological tissue sections on grids for transmission electron microscopy (TEM) is labor-intensive, time-consuming and error-prone. Conventional droplet staining methods require multiple manual handling steps which can easily result in dropped, damaged or lost grids. Users preparing several grids at once face additional challenges: extra effort is demanded to avoid mix-ups, and consistent staining timing is difficult to achieve. Commercial or makeshift devices may be used to process multiple grids, but often require numerous manual grid-handling steps and/or consume larger amounts of toxic reagents.

A new capsule-based system, now available, makes grid staining easier and more efficient. Each grid is typically handled only twice in the process, reagent consumption is reduced and identifying capsule labels provide secure tracking of every specimen.

One or two grids are held securely in each enclosed, barcode-labeled mPrep/g™ capsule for all staining procedures, as well as for secure archival storage (Figure 1). Manual handling is typically required only to insert grids into the capsule at the microtome and again for removal to place them into the TEM stage. Capsules are conveniently stored in mPrep capsule grid boxes, along with any associated resin blocks prepared in mPrep/s™ capsules.

A standard laboratory pipettor, such as the Pipetman P200, is used to easily, safely and precisely deliver stains and rinses directly to grids contained in mPrep/g capsules (Figure 2). Multichannel pipettors allow parallel processing of multiple grids, thus assuring consistent timing for each step of the protocol and greatly reduced labor as compared with manual droplet staining.

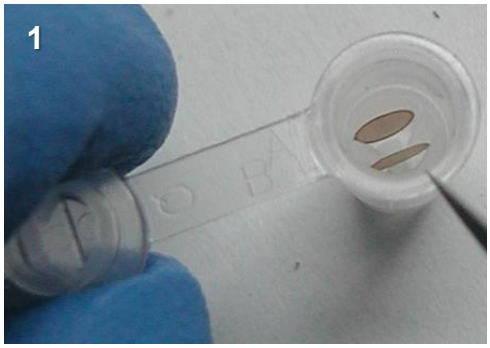

Figure 1: Two grids are inserted into slots “A” and “B” of an mPrep/g capsule.

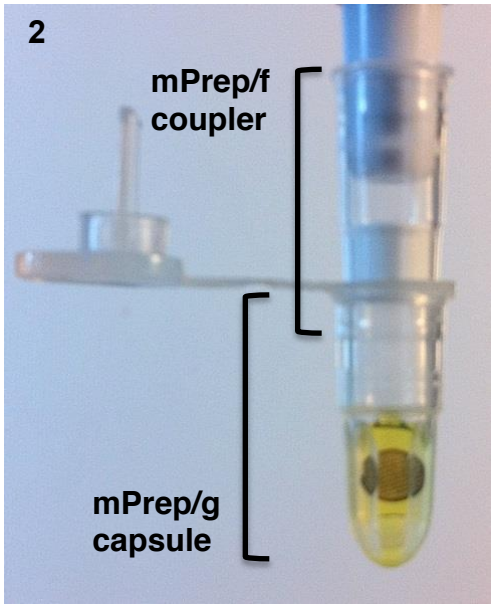

Figure 2: Stain covers grids in mPrep/g capsule connected to a single-channel pipettor with an mPrep/f filter coupler. The capsule contains 35 µl of uranyl acetate stain. (Barcode label was removed to show detail.)

Specimen Preparation

Rat kidney was processed as detailed in applications note AN501. Vampire bat brain was processed similarly. Briefly, tissues were fixed in Karnovsky’s glutaraldehyde-formaldehyde, postfixed with OsO_4, en bloc uranyl acetate stained, dehydrated in acetone and embedded in Epon-Spurrs resin. Specimen preparation took place entirely in mPrep/s™ capsules ensuring traceability.

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Sectioning

Ultrathin sections were prepared using a Leica U67 or Reichert Ultracut E ultramicrotome with a diamond knife and collected on 200 mesh thin bar copper grids. Two grids were stored in each mPrep/g capsule (Figure 1) in slots A and B. Capsules were placed in a labeled capsule grid box (Figure 4) until staining.

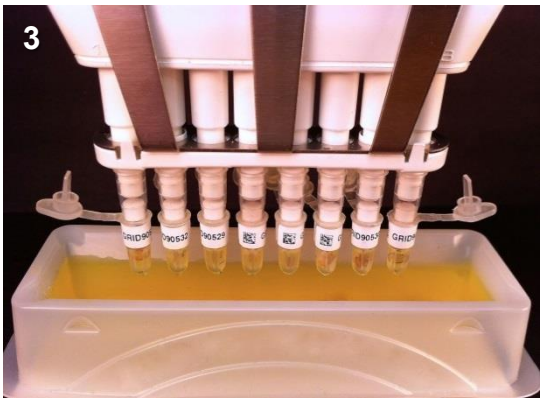

Figure 3: Eight barcode-labeled mPrep/g capsules with grids fitted to an 8-channel pipettor using mPrep/f filter couplers. Capsules contain stain drawn from reagent reservoir.

Staining

Eight mPrep/g capsules containing 16 prepared grids were removed from the capsule grid storage box and attached to an 8-channel pipettor (Pipetman Neo P8X200N) pre-fitted with eight mPrep/f™ couplers. All aspiration and dispense volumes were 35 µl:

1. A reagent reservoir was filled with sufficient freshly filtered 2.5% uranyl acetate in 50% ethanol to enable pipetting the stain into the capsules. Immediately thereafter the reagent was aspirated into mPrep/g capsules (Figure 3) and incubated for 14 minutes with capsules resting in the reservoir. A lab stand was used to stabilize the pipettor. Reservoir and mPrep/g capsules were kept dark by covering with aluminum foil.
2. Uranyl acetate was dispensed to waste and the grids rinsed with dIH_2O a total of 24 times, as follows: a) rinse water was aspirated into capsules, held for 1-2 seconds and dispensed to waste; b) after every 8 rinses, the reservoir was refilled with fresh dIH_2O. All 24 rinses were accomplished in about one minute.*
3. Fresh Reynolds' lead citrate was aspirated into capsules from a clean reservoir immediately after filling it. The stain was incubated on the grids for 9 minutes.
4. Lead citrate was dispensed to waste and the grids were rinsed 24 times with dIH_2O, as described above.
5. Rinse water was expelled from the capsules. Capsules were then removed from the pipettor and separated from the mPrep/f couplers.
6. Remaining water was wicked away from the grids by inserting a wedge of filter paper into the capsule and touching the paper to where the grids contact the inside of the capsule. Grids were then air dried until TEM imaging.

*By design, mPrep/g capsule volume is small to reduce stain consumption and facilitate easy grid insertion and removal. Thus, many rinses are required; the number of rinses may be increased with no deleterious effects.

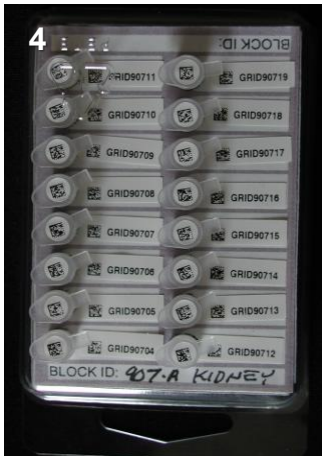

Figure 4: mPrep capsule grid box holds up to 32 grids in 16 labeled mPrep/g capsules.

Results

Uniform and precipitate-free staining is evident in both sets of TEM images. Figure 5 shows rat kidney imaged with a Philips CM120 TEM at 80 KeV. Figure 6 shows brain from a vampire bat imaged with a Hitachi H-7600 TEM at 80 KeV.

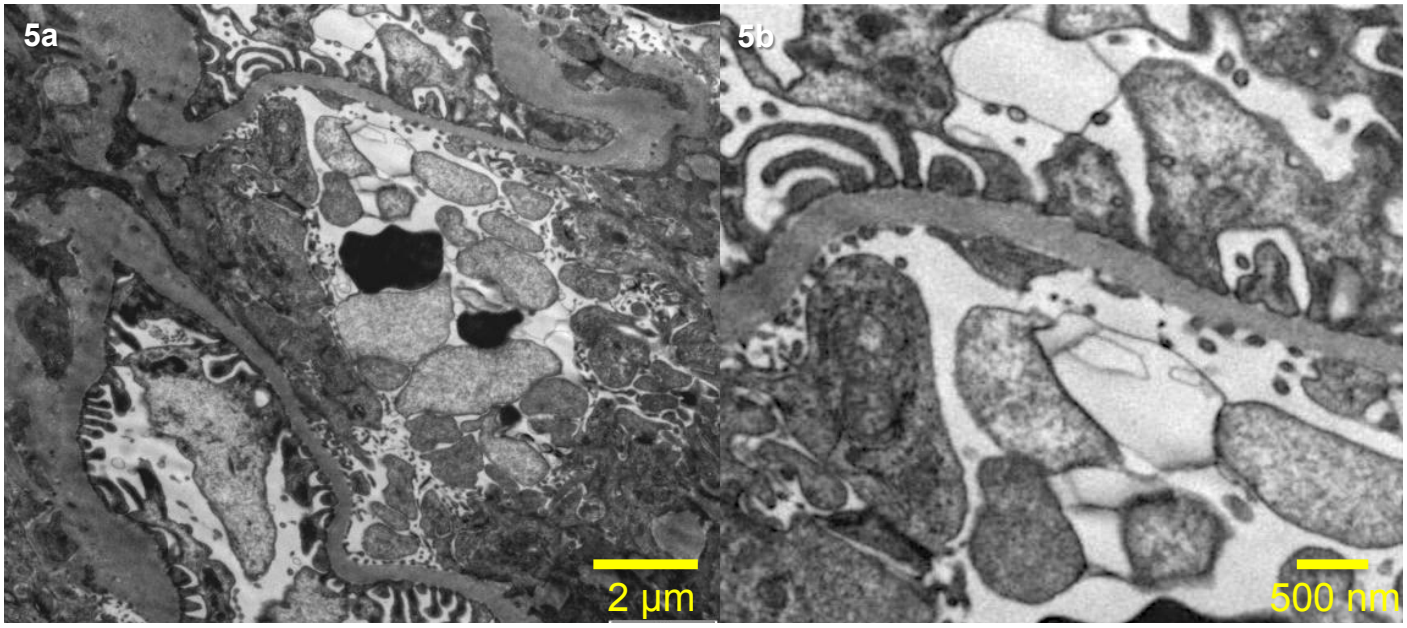

Figure 5: Rat kidney at survey and higher magnification.

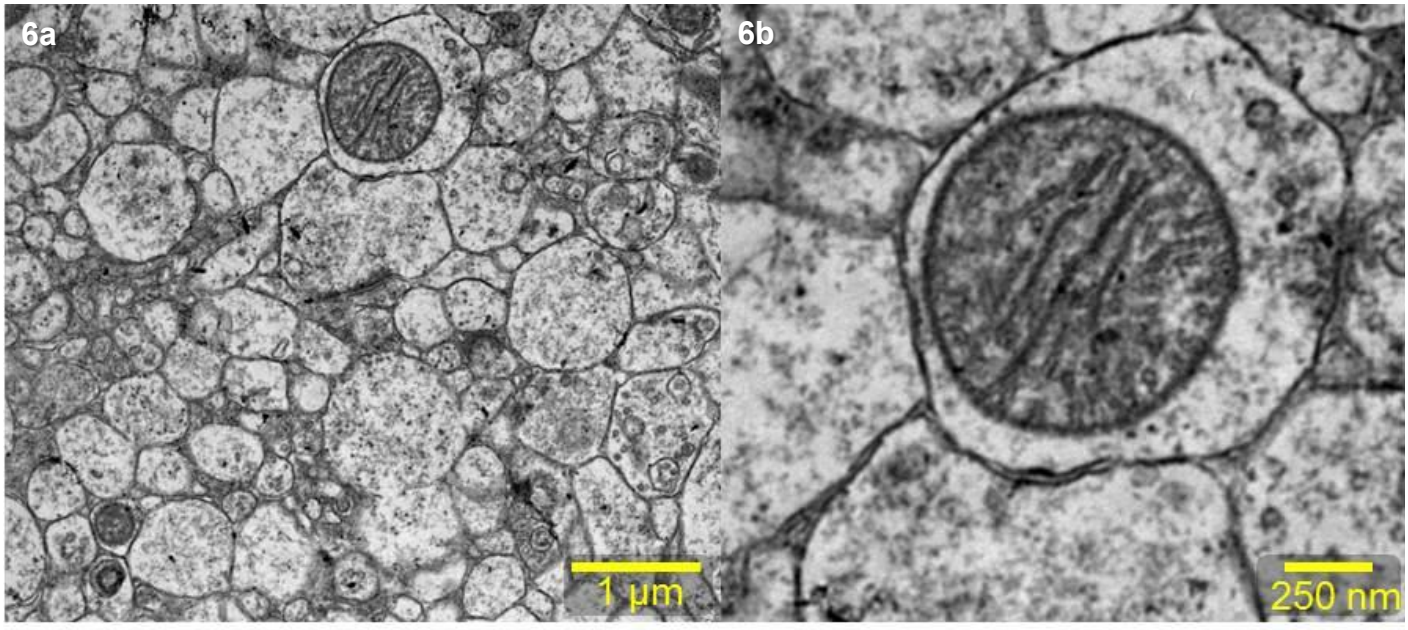

Figure 6: Vampire bat brain at survey and higher magnification.

Acknowledgement

Vampire bat brain was provided courtesy of Craig Radi of the Wisconsin Veterinary Diagnostic Laboratory.

Conclusions

Overall quality of staining using mPrep/g capsules was comparable to conventional droplet staining. The capsule method, however, provided distinct efficiency advantages:

- Labor savings in grid handling — each grid required only two "touches" from microtome to imaging. Possibility of dropped grids was greatly reduced.
- Reduced potential for grid damage or loss, as a result of less grid handling. Fewer repeats translates to greater laboratory efficiency.
- Reagent consumption and corresponding disposal costs were reduced. The mPrep/g capsule uses just 35 µl of reagent to process two grids.
- Capsule labeling system improves sample tracking and minimizes record-keeping time.
- Capsule grid box enabled storage of grids and associated resin blocks in one place for more efficient archival storage and retrieval.

Processing multiple grids was significantly easier, with greater processing consistency:

- Using a multichannel pipettor was highly efficient. Once grids are loaded into capsules, no additional time or user effort is required to scale up from a single grid to 24 grids (using a 12-channel pipettor).
- Even processing many grids at once, there is no risk of mixing up grids contained in labeled mPrep capsules.
- Every grid received absolutely uniform stain exposure and rinsing, removing a variable present in droplet staining methods.

Capsule-based processing offered additional convenience and safety:

- Grids in enclosed capsules are not exposed to ambient air, thus eliminating the need for NaOH pellets to prevent precipitation. This convenience saves time and money.
- Enclosed capsules and smaller reagent volumes can reduce potential exposure to toxic chemical stains.

Overall, this easy and efficient system for staining biological tissue provides excellent quality, consistency and safety with minimal hands-on time.

Ordering Information

Product #	Item Description/Catalog Information
G1600	16 mPrep/g capsules & 16 label sets in capsule/grid storage box
F1601	16 mPrep/f standard pore filter couplers in capsule/grid storage box
B96S	mPrep/bench Model 96S silicone rack for mPrep capsules, 96-well
R1550	15ml reagent reservoirs, non-sterile, HDPE, 50/PK
KIT- xxx	Custom starter kits with mPrep capsules and accessories (please inquire)

AN502 rev1