Capsule-based Processing of Biological Tissue for TEM: Simple, Efficient, Reproducible, Secure

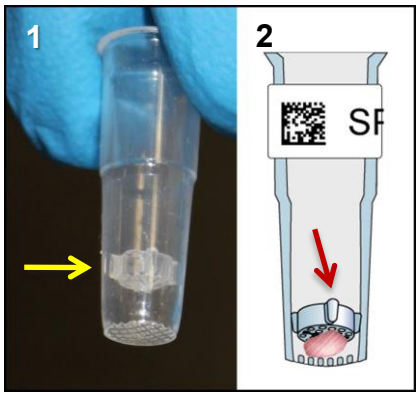
mPrep™ System

Applications Note #501

Introduction

Preparing biological tissue for transmission electron microscopy (TEM) using conventional methods is time-consuming, labor-intensive and error-prone. Scores of individual liquid and specimen transfer steps are required for fixation, washes, dehydration, infiltration and polymerization. Conventional processing methods demand great manual dexterity and scrupulous attention to detail. Keeping track of individual specimens is difficult. Reagent consumption often exceeds minimum requirements.

A simple, efficient new system for end-to-end processing of biological tissue for TEM is now available. Specimens are inserted into secure, barcode-labeled mPrep/s™ processing capsules. With standard laboratory pipettors, users can quickly and easily perform all required fluid exchanges in a logical, easy-to-follow procedure. The specimen remains in the capsule for final embedding, thus eliminating the messy transfer to an embedding mold. The system is scalable, allowing users to efficiently process one specimen using a single channel pipettor, or dozens with a multichannel pipettor or standard liquid handling lab robotics.



Figures 1 and 2: Photograph and diagram of mPrep/s capsule. Arrows show adjustable mPrep/s screen that entraps specimen, as illustrated in cutaway diagram.

Procedure

Rat kidney was perfused, excised, immersed in Karnovsky's fixative and refrigerated. Specimens were then cut into circa 1 mm^3 pieces and inserted into labeled mPrep/s capsules (Figures 1 & 2). Each specimen was secured with the capsule's removable and position-adjustable screen (Figures 1 & 2). Capsules were then attached to a multichannel pipettor fitted with mPrep/f™ couplers (Figure 3) to protect the pipettor from accidently drawing reagents into the pipette mechanism. Each mPrep/s capsule was individually labeled with specimen identification (Figures 2 & 3).

For sectioning, the mPrep/s capsule with an epoxy-embedded specimen was directly clamped into the microtome chuck, where it was trimmed through the capsule, faced and sectioned (Figure 4).

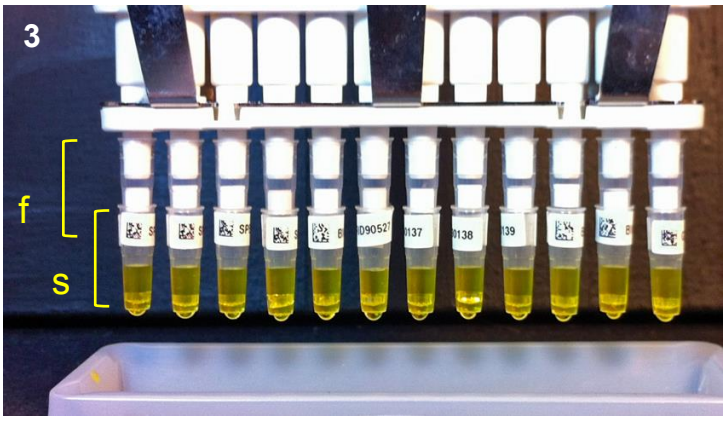


Figure 3: A dozen barcode-labeled mPrep/s capsules filled with 100 µl of uranyl acetate en bloc stain from reagent reservoir. The mPrep/s capsules (s) are connected to a multichannel pipettor with mPrep/f filter couplers (f).

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Experimental Protocol

Tissue was processed in mPrep/s capsules attached to a 12-channel pipettor (Pipetman Neo P12X200N) by simultaneously drawing 100 µl of the following reagents from a reagent reservoir into the specimen capsule:

1. 3 x 5 minute washes in Sorensen's sodium phosphate buffer (to rinse out Karnovsky's fixative)
2. 1% OsO_4 for 1 hour
3. 3 x 10 minute washes in dIH_2O
4. 1% aqueous uranyl acetate for 1 hour
5. 3 x 10 minute washes in dIH_2O
6. 1 each 15 minute washes of 20%, 50%, 70%, and 90% acetone
7. 3 x 20 minute washes in 100% acetone
8. 1:2 Epon/Spurr's:acetone infiltration for 1 hour (see Resin Formula below)
9. 1:1 Epon/Spurr's:acetone infiltration for 1 hour
10. 2:1 Epon/Spurr's:acetone infiltration for 1 hour
11. 100% Epon/Spurr's resin infiltration for 12 hours at room temperature
12. mPrep/s capsules filled with resin were then inserted into an mPrep/bench™ silicone rack and transferred to a 60° oven for 24 hour in-capsule polymerization
13. Polymerized blocks, still contained in the mPrep/s capsule, were directly chucked into the microtome, faced through the capsule and sectioned.

Figure 4: mPrep/s capsule with epoxy block directly clamped in microtome chuck, trimmed through capsule and faced for sectioning.

Light Microscopy

Thick 0.5 µm sections were prepared using a Leica UC7 ultramicrotome, collected on glass slides, stained with polychrome I (methylene blue, azure II, 10% methanol 10% glycerol), and examined with an Olympus BH2 microscope with Nikon 700 DSLR camera (Figure 5).

Transmission Electron Microscopy

Ultrathin 70 nm sections were collected on 200 mesh Cu grids. Two grids were inserted into barcode-labeled mPrep/g™ capsules for post-staining with 35 µl per capsule (see applications note AN502) using:

1. 2.5% uranyl acetate in 50% ethanol for 14 minutes, followed by multiple water rinses,
2. Reynold's lead citrate for 9 minutes, followed by multiple water rinses, and in capsule blotting.
3. Grids were stored in mPrep/g capsules until imaging with a Philips CM120 TEM at 80 keV (Figure 6).

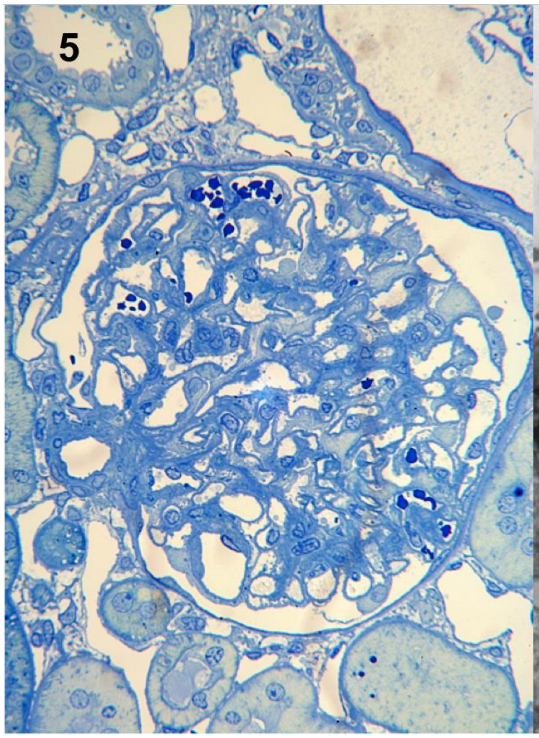

Figure 5: Rat kidney section stained with polychrome I and imaged with light microscopy.

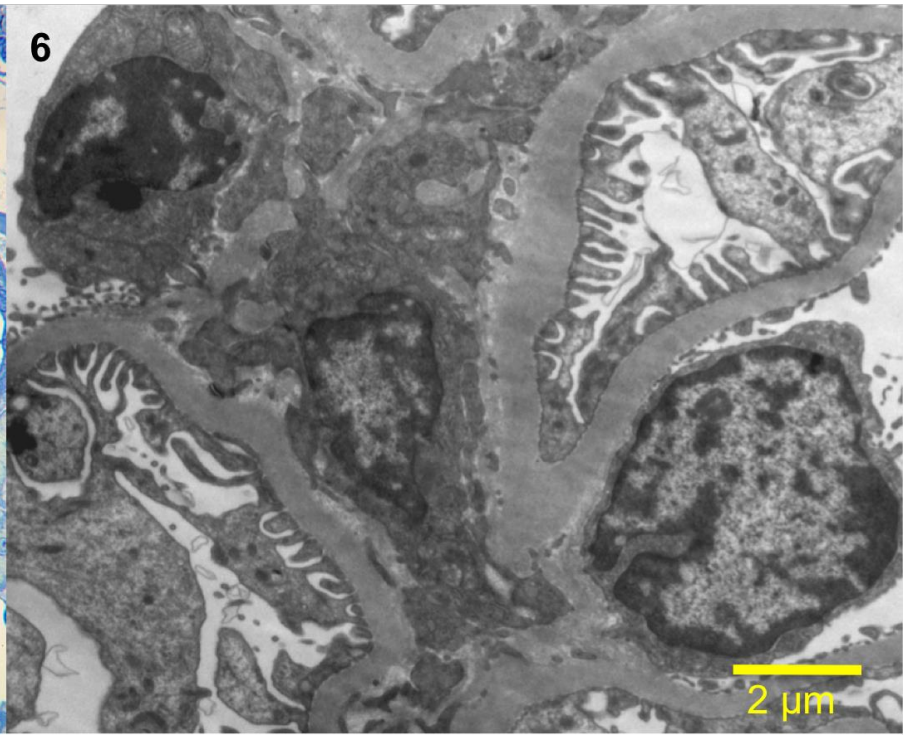

Figure 6: TEM image of thin section, 70 nm thick, from same block as light micrograph.

Results and Conclusions

The overall ultrastructure preservation of rat kidney was comparable to conventional manual processing techniques using vials and manual reagent delivery (and grid droplet staining), but offered distinct advantages in the preparation method:

- Less processing time and effort, due to simultaneous reagent delivery to all specimens
- Better traceability, due to continuous labeling
- Elimination of specimen transfers to embedding molds and removal of blocks from molds
- Significantly lower reagent consumption

Resin Formula

10.0 g vinyl cyclohexane dioxide
6.0 g DER 736 epoxy
26.0 g nonenyl succinic anhydride
0.3 g dimethylaminoethanol
25.0 g Embed-812
13.0 g dodenyl succinic anhydride
12.0 g Nadic methyl anhydride methyl-5-norbornene 2,3-dicarboxilic anhydride
0.53 g 2,4,6-Tri(dimethylaminoethyl)phenol

Ordering Information

Product #	Item Description/Catalog Information
S0812	8 mPrep/s capsules, 12 screens, 8 label sets in capsule/grid storage box
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
TL100	mPrep/s insertion tool
R1550	15ml reagent reservoirs, non-sterile, HDPE, 50/PK
KIT- xxx	Starter kits with mPrep capsules and accessories including Gilson Pipetman (various custom kits available — please inquire)