Fast, Automated Tissue Processing of Mammalian Kidney for TEM with the mPrep™ ASP-1000

mPrep™ System

Applications Note #601

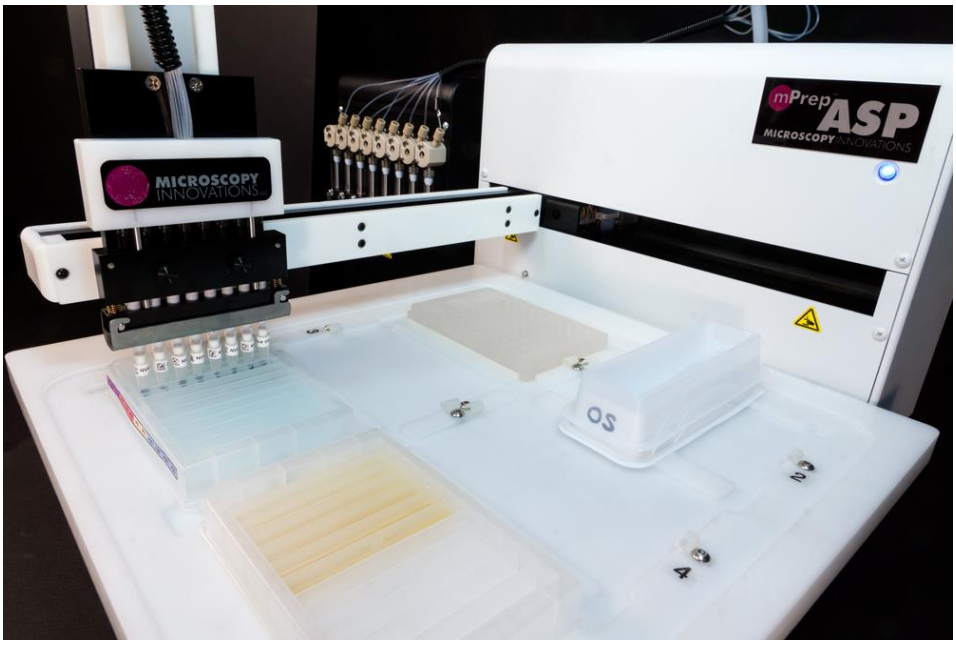

Figure 1: ASP-1000 setup with mPrep/s capsules and reagent plates.

Introduction

Sample preparation of biological tissue for TEM is time-consuming and labor intensive, often presenting a major bottleneck in electron microscopy laboratories. Microwave methods were introduced to speed up tissue processing by accelerating chemical reactions. These methods, however, require frequent manual intervention, thus remaining labor intensive. Automated EM tissue processors were developed to reduce labor, but do little to reduce total elapsed processing time. These conventional processors have further disadvantages that include requiring tedious pre-run assembly (of baskets, ring holders and stacking rings), manual transfer of syrupy resin-infiltrated specimens to embedding baskets or molds, and time-consuming, messy post-run disassembly and cleanup.

Here we present a fast method of processing tissue for TEM, using the mPrep™ ASP-1000 Automated Specimen Processor and highly efficient rapid-agitation mixing. In less than one hour, this system processes mammalian kidney specimens from postfixation rinse through resin infiltration. The tissue specimens held in mPrep/s™ processing capsules (Figure 2) do not require transfer to separate embedding molds for polymerization. Specimens are touched only once, when placed into the capsules, and remain in the traceable, barcode-labeled capsules throughout processing, embedment, and sectioning.

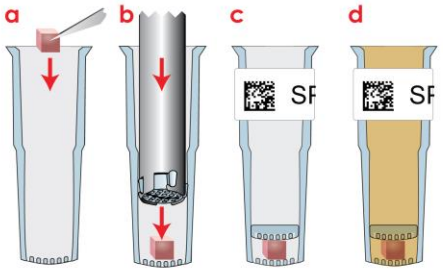

Figure 2: One-touch processing. After placing specimen in an mPrep/s capsule (a), no additional touches are needed. It is secured by a removable top screen (b), labeled with barcode for easy tracking (c), and embedded in the same capsule (d).

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Methods and Materials

Fresh mammalian kidney samples (~2-mm cubes) were placed into labeled mPrep/s capsules, which were placed into correspondingly labeled 1.5-ml microcentrifuge tubes for fixation. The tubes were filled with Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde, 0.1 M phosphate buffer) and fixed overnight (Figure 3) prior to automated processing.

Standard format, automation-compatible microplate vessels were used for ASP-100 processing. Reagents (including Embed 812 resin) were pre-dispensed into two 12-channel polypropylene reagent reservoirs, as shown in Figure 1 and on the Plate Map in Figure 5 (next page). For safety and ease of disposal, osmium tetroxide (OsO_4) was placed in its own reservoir. All filled reagent reservoirs and an empty mPrep/bench™ 96-well silicone rack (to receive capsules for final polymerization) were placed on the deck. The ASP-1000 was operated in a ventilated enclosure to provide a clean environment and control hazardous fumes.

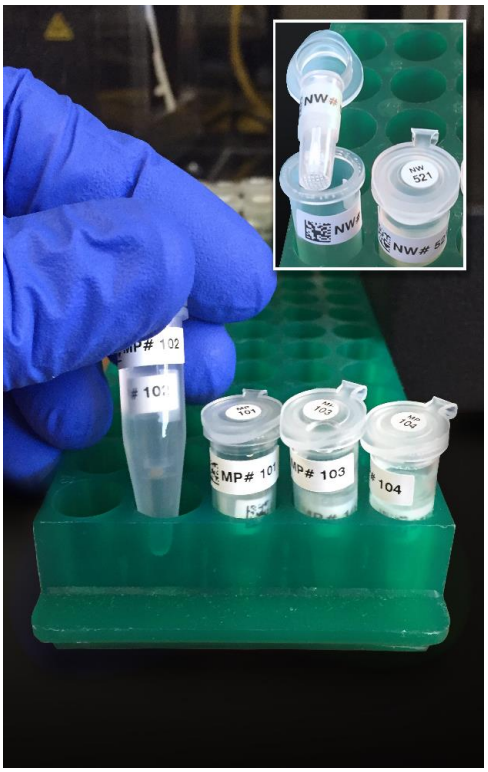

Figure 3: Fixation prior to automated processing. Specimens in mPrep/s capsules fit inside barcode-labeled microcentrifuge tubes for overnight fixation.

Eight mPrep/s capsules containing glutaraldehyde-fixed kidney specimens were mounted on the ASP-1000 (Figure 4) before initiating the 45-minute processing program (Figure 5, Automation Protocol). Rapid processing, achieved by fluid exchanges occurring every half second, constantly delivered fresh reagent to the specimens. For example, in Step 17, 100% resin was exchanged 600 times in 5 minutes. No operator intervention was required.

When all reagent steps were completed, the ASP-1000 moved the capsules to the mPrep/bench silicone rack. The auto-processor then signaled the operator to eject the capsules into the rack. The operator then added a small volume of resin to fill each capsule, and placed the rack in a 60°C oven for polymerization.

Capsules containing polymerized specimens were mounted directly in the microtome chuck, trimmed, and sectioned. Imaging was performed with electron microscopy. Ultrastructural preservation of the kidney specimens was excellent, and produced superb image quality (Figure 6).

Reagent cleanup consisted only of moving the solvent and resin reservoirs (Plates 5 and 6) to the back of a hood, to evaporate the acetone and alcohol, and separately discarding the osmium reservoir into appropriate waste. On the next day, the resin remaining in Plate 6 was polymerized in an oven and discarded.

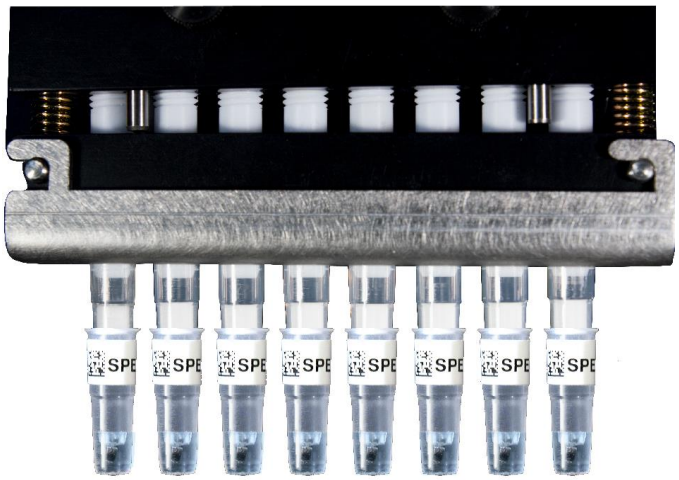

Figure 4: mPrep/s capsules mounted on ASP-1000.

Plate Map

Deck Position/Labware/Reagents

	Position 1	Position 2
Col.	mPrep/bench rack	Single Reservoir
6	empty	Osmium

	Position 3	Position 4
Col.	not used	not used
n/a		

	Position 5	Position 6
Col.	12-Column Reservoir	12-Column Reservoir
1	Fix rinse 1- 0.1M PB	25% resin
2	Fix rinse 2- 0.1M PB	50% resin
3	Fix rinse 3- 0.1M PB	75% resin
4	Os rinse 1 (H20)	100% resin
5	Os rinse 2 (H20)	100% resin
6	50% ethanol	100% resin
7	70% ethanol	
8	90% ethanol	
9	95% ethanol	
10	100% ethanol	
11	Acetone	
12	Acetone	

Automation Protocol

Fast processing/mPrep™ ASP-1000

Step	Reagent	Mins	Secs	Plate	Col.	Fluid exchanges
1	Fix rinse 1	0.5	30	5	1	60
2	Fix rinse 2	0.5	30	5	2	60
3	Fix rinse 3	0.5	30	5	3	60
4	Osmium	5	300	2	6	600
5	Os rinse 1	0.5	30	5	4	60
6	Os rinse 2	0.5	30	5	5	60
7	50% ethanol	0.5	30	5	6	60
8	70% ethanol	0.5	30	5	7	60
9	90% ethanol	0.5	30	5	8	60
10	95% ethanol	0.5	30	5	9	60
11	100% ethanol	2	120	5	10	240
12	Acetone	2	120	5	11	240
13	Acetone	2	120	5	12	240
14	25% resin	5	300	6	1	600
15	50% resin	5	300	6	2	600
16	75% resin	5	300	6	3	600
17	100% resin	5	300	6	4	600
18	100% resin	5	300	6	5	600
19	100% resin	5	300	6	6	600
Total minutes:		45.5				

PROTOCOL MUST BE RUN IN VENTILATED ENCLOSURE OR HOOD!

Figure 5: Reagent layout on auto-processor deck (left) and automation protocol (right).

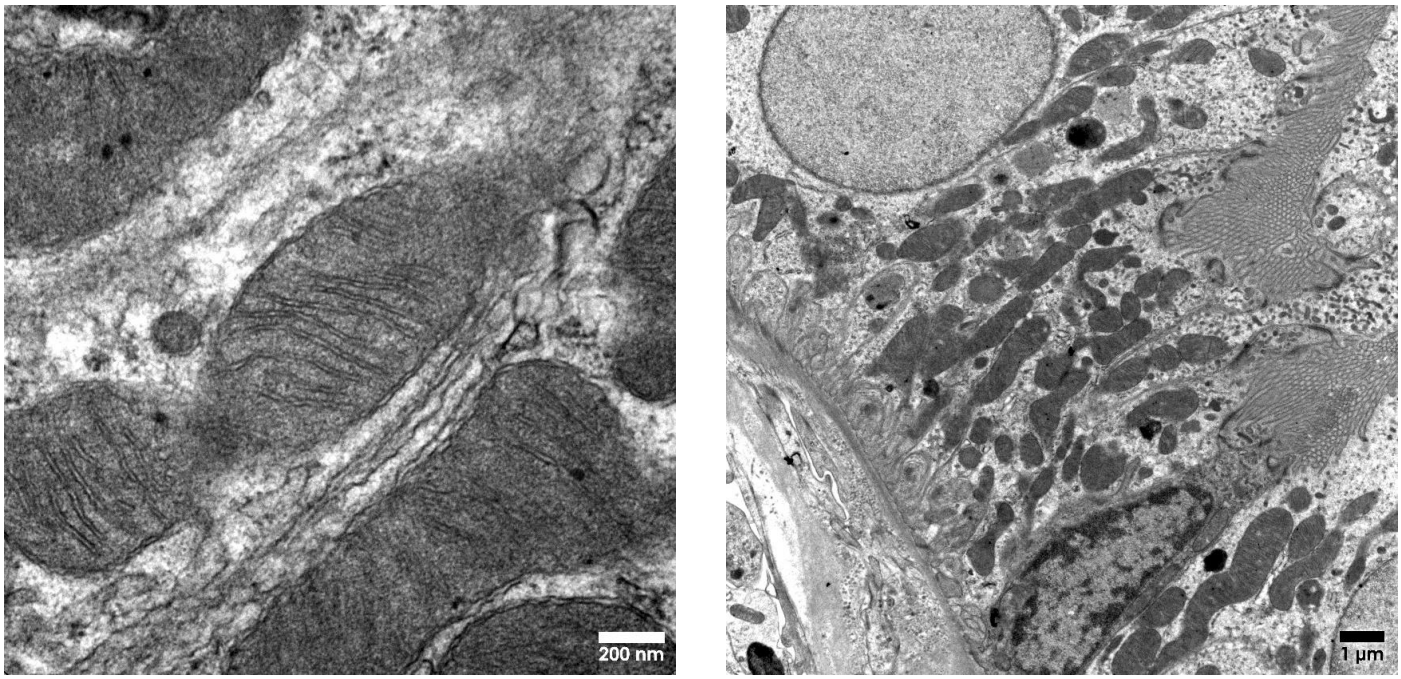

Figure 6: TEM images of mammalian kidney specimens.

Results and Discussion

The mPrep ASP-1000 Automated Specimen Processor saved significant time and reduced manual labor in processing mammalian kidney specimens. Ultrastructure was well preserved and imaging results were excellent.

Compared with microwave methods and other automated tissue processors, the ASP-1000 offered these distinct advantages:

- Total reagent processing time was significantly reduced. Only 45 minutes was required from postfix rinse through 100% resin infiltration.
- No operator intervention or specimen handling was required at any time during reagent processing.
- Handling resin-infiltrated specimens and transfer to embedding molds was eliminated.
- Specimens were touched only once, to place them in labeled mPrep/s capsules, where they remained through embedding and sectioning. Thus, labor and the potential for error were greatly reduced.
- Reagent setup was easy and logical. Cleanup was simple due to the use of disposable reagent reservoirs.
- The ASP-1000 eliminated the frequent technician involvement needed in microwave processing methods, and the complicated assembly and disassembly steps typical for other automated tissue processors.

Conclusions

Compared with other methods of preparing tissue specimens for TEM, use of the ASP-1000 significantly reduced total processing time and the hands-on effort required of technicians. Automated processing in capsules achieved consistent results with less effort and fewer opportunities for sample damage or mix-up.

Ordering Information

Product #	Item Description/Catalog Information
41000	mPrep ASP-1000 Automated Specimen Processor
22200	mPrep/s capsules in storage box - 8 capsules, 12 screens, 8 blank label sets
22500	mPrep/s capsules - bulk pack: 96 capsules, screens & blank label sets
31500	mPrep/f30 Standard filter-couplers in capsule storage box, 16/pk
34000	mPrep/bench 96-well rack, silicone
52501	12-channel reagent reservoir, polypropylene, sold by EACH, 25 each/case
52001	R15-50HDPE - 15ml Reagent Reservoirs, non-sterile, HDPE, 50/pk
32010	mPrep/s Insertion Tool

Benefits of ASP-1000 Processing in mPrep/s™ Capsules

- Fast processing offers quicker results.
- Simple setup and cleanup saves effort.
- Technicians are more productive when freed from frequent manual intervention or complicated setup/cleanup procedures.
- The simple-to-use ASP-1000 is supplied with many ready-to-use protocols.
- Easily customizable COBRA control software allows an unlimited number of processing steps.
- The PC-based software can send text messages to operators for any step needing operator involvement, such as when processing is complete.
- The ASP-1000 deck holds up to six standard microplates or reservoirs, allowing up to 72 reagent or rinse positions.
- Dispensing reagents from microplates reduces consumption and enables up to eight variable conditions.
- Automation provides precise reagent control and uniform processing times across multiple samples.
- Specimen handling is reduced to “one touch” with mPrep/s capsules.
- Barcode or alphanumeric labeling of capsules reduces error potential, simplifies sample management, and enables GLP compliance.
- Patented mPrep/s capsules allow users to orient a specimen during placement into the capsule without further manipulation.
- Automated processing in mPrep capsules provides scientists with consistent results day-in-and-day-out.

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