



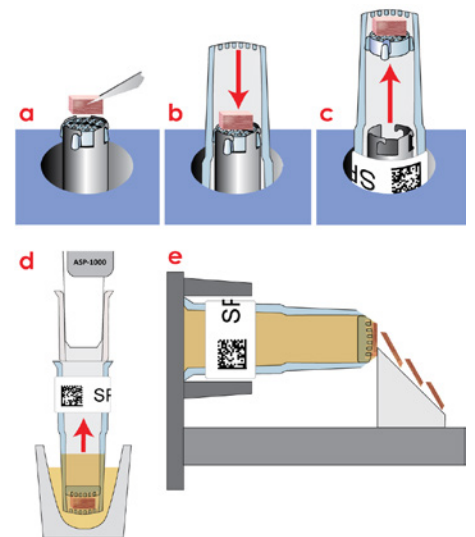
Figure 1: ASP-1000 setup with mPrep/s capsules and reagent plates.

## Introduction

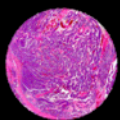
Sample preparation of biological tissue for TEM is time-consuming and labor intensive, often presenting a major bottleneck in electron microscopy laboratories. Methods utilizing microwaves can speed up tissue processing, but still require frequent manual intervention, thus making them labor intensive.

Conversely, automated EM tissue processors were developed to save labor, but most do little to reduce total elapsed processing time. In addition, many have further disadvantages such as 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 muscle tissue for TEM that uses the mPrep™ ASP-1000 Automated Specimen Processor (Figure 1). In just over two hours, mammalian muscle specimens are processed from postfixation rinse through resin infiltration. The tissue specimens held in mPrep/s™ capsules for processing do not require transfer to embedding molds before placing in an oven for polymerization. Specimens are handled only once, when placed into the capsules, and remain in the barcode-labeled capsules throughout processing, embedding, and sectioning (Figure 2).



**Figure 2: One-touch processing.** Using the mPrep/s Workstation (blue), you can load a specimen on screen (a) and secure the oriented specimen in a barcode-labeled capsule (b and c). Then use the ASP-1000 for reagent processing (d), and, after curing resin in capsule, place it directly into the chuck of a microtome for sectioning (e).



**Methods and Materials**

Muscle tissue from neonatal mice was perfused, excised, immersed in fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer), and refrigerated by University of Wisconsin – Madison (UW) researchers. Later, specimens were cut into ~2x2x4-mm rectangular pieces and inserted into labeled mPrep/s capsules. The mPrep/s Workstation was used to ensure proper orientation (Figure 2) and enable longitudinal sectioning of the muscle cells.

Reagents, including Embed 812 resin, were pre-dispensed into two 12-column polypropylene reagent reservoirs. For safety and ease of disposal, osmium tetroxide (OsO<sub>4</sub>) was placed in its own reservoir. All filled reagent reservoirs and an empty mPrep/bench™ 96-well silicone rack (to receive capsules for polymerization) were placed on the ASP-1000 deck (Figure 3). The ASP-1000 was operated in a ventilated enclosure to control hazardous fumes.

Eight mPrep/s capsules containing glutaraldehyde-fixed muscle specimens were mounted on the ASP-1000 before initiating the 133-minute processing program (Figure 4). Fluid exchanges every two seconds constantly delivered fresh reagent to the specimens. Osmium was added to and removed from the specified reservoir shortly before and after use to ensure operator safety. To minimize evaporation, acetone was added immediately before initiating the program.

Plate Map																													
Deck Position/Labware/Reagents																													
	<table border="1"> <tr> <th>Position 1</th> <th>Position 2</th> </tr> <tr> <td>mPrep/bench rack</td> <td>Single Reservoir</td> </tr> <tr> <td>6 empty</td> <td>Osmium</td> </tr> </table>	Position 1	Position 2	mPrep/bench rack	Single Reservoir	6 empty	Osmium																						
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Automation Protocol						
Fast Processing/mPrep™ ASP-1000						
Step	Reagent	Time (min)	Plate	Column	Fluid exchanges	Delay (ms)
1	Fix Rinse 1	2.3	5	1	60	0
2	Fix Rinse 2	2.3	5	2	60	0
3	Fix Rinse 3	2.3	5	3	60	0
4	Osmium	35	2	6	600	500
5	Os Rinse 1	2.3	5	4	60	0
6	Os Rinse 2	2.3	5	5	60	0
7	50% Ethanol	2.3	5	6	60	0
8	70% Ethanol	2.3	5	7	60	0
9	90% Ethanol	2.3	5	8	60	0
10	95% Ethanol	2.3	5	9	60	0
11	100% Ehtanol	4.6	5	10	120	0
12	Acetone 1	4.6	5	11	120	0
13	Acetone 2	4.6	5	12	120	0
14	25% Resin	10.5	6	1	180	500
15	50% Resin	10.5	6	2	180	500
16	75% Resin	10.5	6	3	180	500
17	100% Resin	10.5	6	4	180	500
18	100% Resin	10.5	6	5	180	500
19	100% Resin	10.5	6	6	180	500
Total Time		132.5	2 hours 12.5 minutes			

**PROTOCOL MUST BE RUN IN VENTILATED ENCLOSURE OR HOOD!**

Figure 3: Reagent layout on auto-processor deck

Figure 4: Automation protocol

When all reagent steps were completed, the ASP-1000 automatically positioned the capsules above the mPrep/bench silicone rack and prompted the operator to eject them 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.

Reagent cleanup consisted simply of moving the solvent and resin reservoirs (Plates 5 and 6) to the back of a fume hood to evaporate the acetone and alcohol, then emptying the osmium reservoir into appropriate waste after depleting with corn oil. On the next day, the resin remaining in Plate 6 was polymerized in an oven and discarded.

Capsules containing polymerized specimens were mounted directly in the microtome chuck (see Figure 2e on page 1). The block face containing longitudinally oriented cells was then trimmed and sectioned (70–90 nm) before imaging thin sections with TEM (Figure 5).

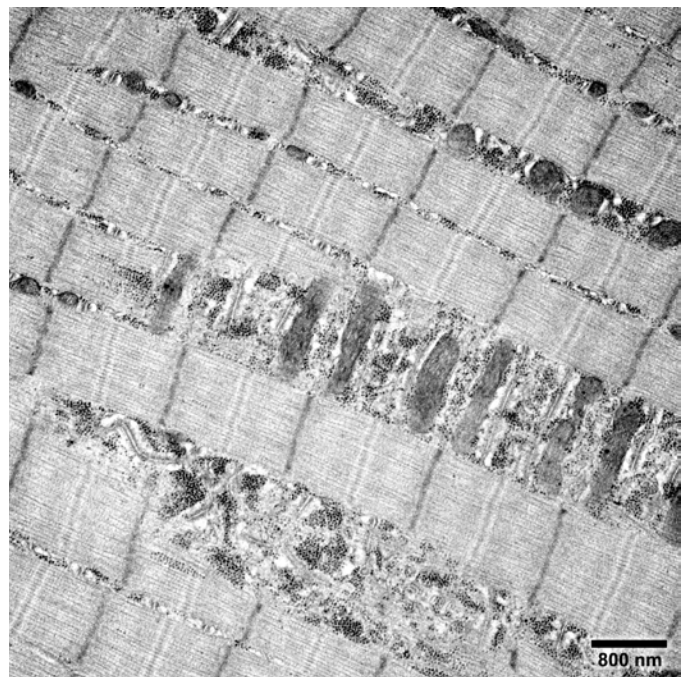
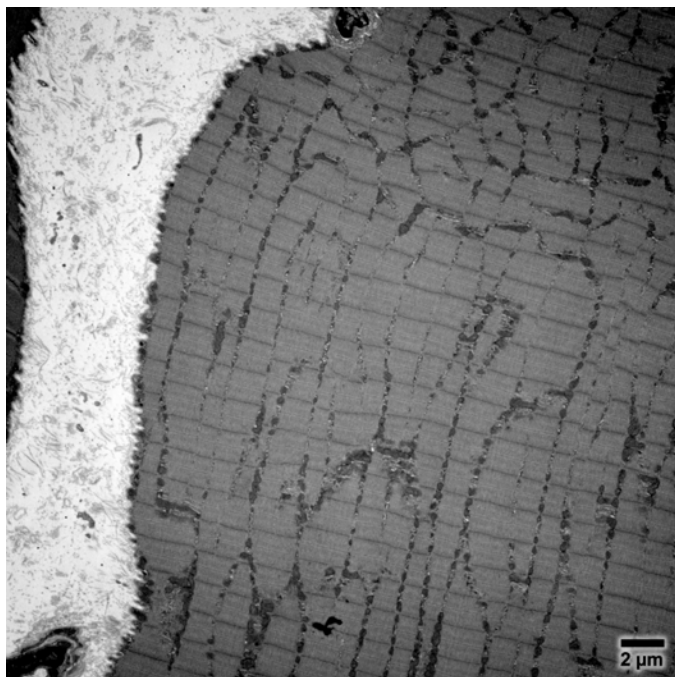


Figure 5: TEM images of muscle tissue specimens.

## Results and Discussion

The mPrep ASP-1000 Automated Specimen Processor saved significant time and reduced effort in processing mammalian muscle specimens.

Ultrastructure—specifically mitochondrial ultrastructure—was well preserved. Imaging results were excellent and suitable for publication, which is anticipated by the UW researchers in Spring 2017.

The ASP-1000 offered these distinct advantages over alternative methods:

- Processing speed was reduced: only 133 minutes was required from postfix rinse through 100% resin infiltration.
- Handling of resin-infiltrated specimens and transfer to embedding molds were eliminated.
- Specimens were handled 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 and cleanup was easy and logical 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.

## Ordering Information

Product #	Item Description/Catalog Information
41000	mPrep ASP-1000 Automated Specimen Processor
42100	mPrep/s Workstation
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.