Automated Preparation of Agar-Embedded Cells for TEM

mPrep™ System

Applications Note #604

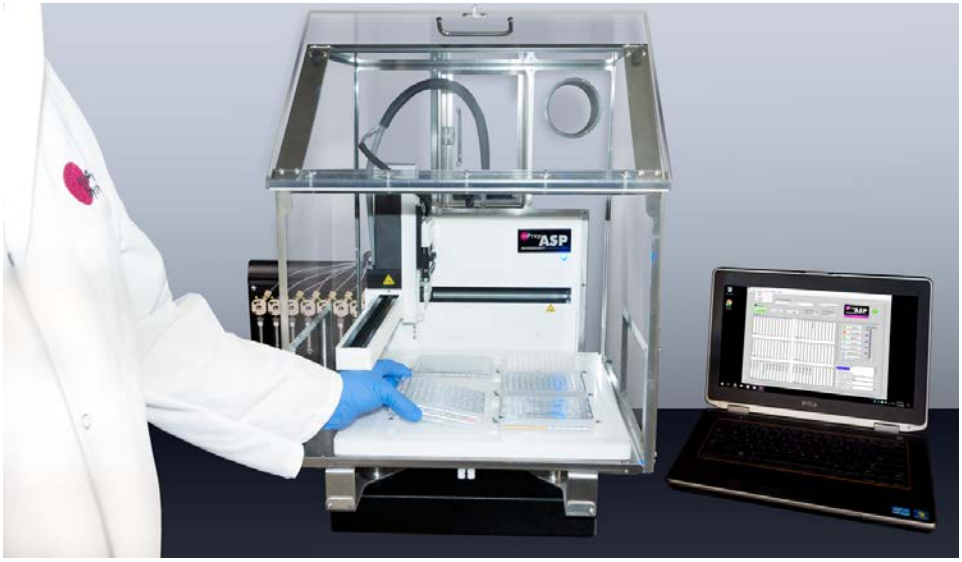

Figure 1: ASP-1000 setup with mPrep/s capsules and reagent plates.

Introduction

Agar embedment is a useful method for turning suspensions of cells into tissue-like blocks for ease of handling during processing for TEM. It is commonly used for yeast, mammalian cells, prokaryotic cells, cilia, and other small specimens. Advantages of embedment include a reduced potential for cell damage or loss and the elimination of multiple time-consuming cell pelleting cycles (centrifugation and resuspension) that are conventionally used to exchange each processing reagent.

Employing a fully-automated specimen processor for the TEM specimen preparation steps after embedment further reduces manual labor and time expended, while providing excellent sample-to-sample and run-to-run consistency, repeatability, and reproducibility.

Here we present a robust method for preparing cell suspensions that begins with fixation of yeast cells in suspension, followed by pelleting and embedding in agar. The agar-embedded cells are then automatically and rapidly processed using the mPrep™ ASP-1000 Automated Specimen Processor (Figure 1).

Methods and Materials

Suspensions containing yeast cells (*S. cerevisiae*) were gently pelleted at 300 x *g* for 3 minutes in 1.5 mL microcentrifuge tubes. The supernatant was

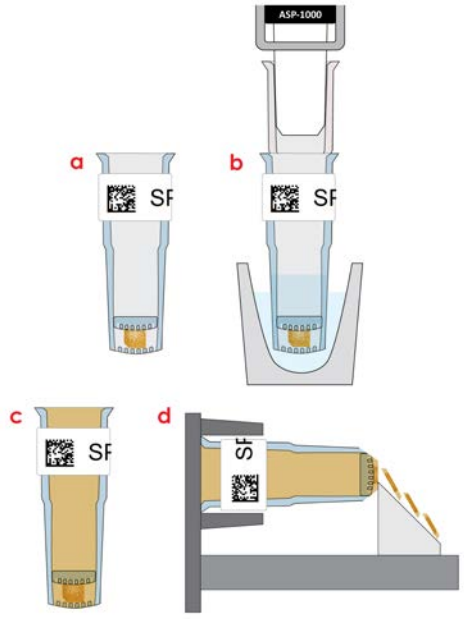

Figure 2: Workflow for an mPrep/s capsule containing an agar-embedded yeast sample. Steps include: a) securing an agar-embedded yeast sample in an mPrep/s™ capsule, b) fluid processing steps on the ASP-1000 instrument, c) resin polymerization, and d) sectioning the block within the capsule with a microtome.

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Plate Map

Deck Position/Labware/Reagents

Position 1	Position 2
mPrep/bench rack	Single Reservoir
empty	Osmium
Position 3	**Position 4**
not used	not used
Position 5	**Position 6**
12-Column Reservoir	12-Column Reservoir
Fix rinse 1 (0.1M PB)	25% resin (Epon 812)
Fix rinse 2 (0.1M PB)	50% resin (Epon 812)
Fix rinse 3 (0.1M PB)	75% resin (Epon 812)
Os rinse 1 (H20)	100% resin (Epon 812)
Os rinse 2 (H20)	100% resin (Epon 812)
50% ethanol	100% resin (Epon 812)
70% ethanol	
90% ethanol	
95% ethanol	
100% ethanol	
Acetone	
Acetone	

Automation Protocol

Fast processing cells/mPrep™ ASP-1000

Step	Reagent	Time (min)	Plate	Column	Fluid Exchanges	Delay (ms)
1	Fix rinse 1	2.5	5	1	60	1000
2	Fix rinse 2	2.5	5	2	60	1000
3	Fix rinse 3	2.5	5	3	60	1000
4	Osmium	25.0	2	6	600	1000
5	Os rinse 1	2.5	5	4	60	1000
6	Os rinse 2	2.5	5	5	60	1000
7	50% ethanol	2.5	5	6	60	1000
8	70% ethanol	2.5	5	7	60	1000
9	90% ethanol	2.5	5	8	60	1000
10	95% ethanol	2.5	5	9	60	1000
11	100% ethanol	5.0	5	10	120	1000
12	Acetone	5.0	5	11	120	1000
13	Acetone	5.0	5	12	120	1000
14	25% resin	6.3	6	1	150	1500
15	50% resin	6.3	6	2	150	1500
16	75% resin	6.3	6	3	150	1500
17	100% resin	6.3	6	4	150	1500
18	100% resin	6.3	6	5	150	1500
	Total time	93.8				

PROTOCOL MUST BE RUN IN VENTILATED ENCLOSURE OR HOOD!

Figure 3: Reagent layout on auto-processor deck (left) and automation protocol (right).

carefully removed by pipetting and the cells were washed by adding 1 mL of 0.1 M phosphate buffer and resuspended by gentle pipetting. After waiting 5 minutes, the suspension was re-centrifuged, the supernatant was aspirated by pipet, and an equal volume of double strength fixative (8% paraformaldehyde, 4% glutaraldehyde in 0.1 M phosphate buffer) was added and mixed by gentle pipetting.

The yeast cells were then fixed overnight at 4°C. The fixative was removed by four repeated washes consisting of pelleting (300 x *g* for 3 minutes), pipetting off the supernatant, and resuspending in 1 mL 0.1 M phosphate buffer for 5 minutes. After the last wash, 4% Type 1 low-melting agarose gel was prepared in a microwave and cooled to 60°C. A volume of agarose solution equal to the final pelleted yeast volume was added to the tube and gently mixed. To expedite gelation, the tubes were cooled on ice.

Once solidified and cooled to room temperature, the gel “plugs” were removed from the centrifuge tubes by cutting off the tip and pushing the gel out through the top. After cutting the gel plugs into 1-mm cubes, the cubes

were placed into eight labeled mPrep™/s capsules (Figure 2a) and immersed in buffer to maintain hydration until ready for processing.

The mPrep/s capsules containing agar embedded cells were mounted on the ASP-1000 before initiating the automated processing program (Figure 3, Automation Protocol). Reagents had been pre-dispensed into 12-column reservoirs for automation, as shown in Figure 3, Plate Map. Processing of eight samples took place automatically in 94 minutes from rinses through 100% resin infiltration.

After completing all reagent steps, the ASP-1000 automatically moved the capsules to the mPrep/bench silicone rack and signaled the operator to eject the capsules into the rack. The operator then added a small volume of resin to fill each capsule to the top (Figure 2b, 2c) and placed the rack in a 60°C oven for overnight polymerization.

Capsules containing polymerized specimens were mounted directly in the ultramicrotome chuck, trimmed, and cut in 70-nm sections (Figure 2d). The sections were placed on Formvar-filmed grids, stained (with uranyl acetate and Reynolds lead citrate), and imaged by TEM at 80 KeV (Figure 4).

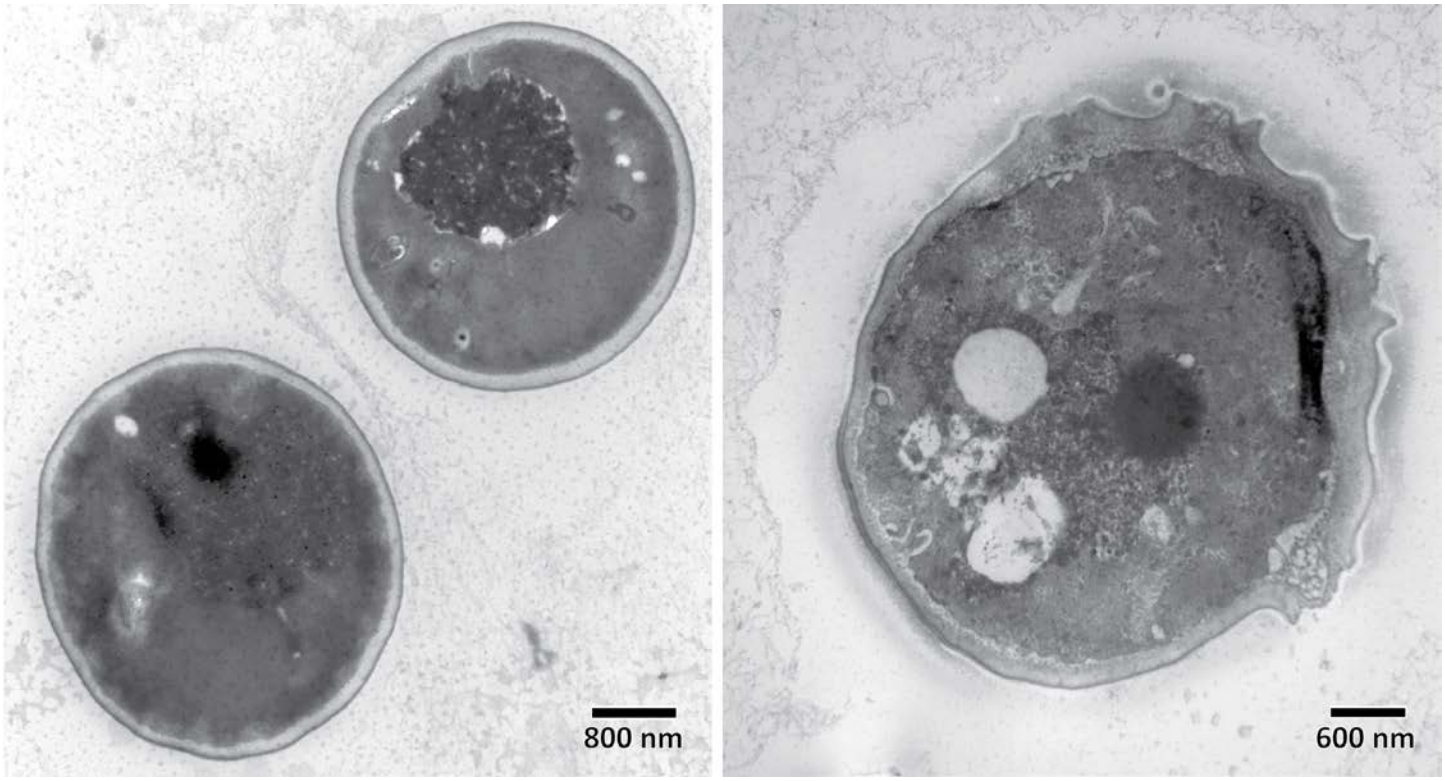

Figure 4: TEM images of prepared yeast cells.

Results and Discussion

Images of cells prepared using the ASP-1000 (Figure 4) demonstrate that the cells are well-preserved and nicely embedded. Agar embedment successfully held yeast pellets together so that the cells could be processed in the same manner as tissue. Embedment of the cells eliminated 18 centrifugation and resuspension cycles that would otherwise have been required for exchanges of TEM reagents following initial fixation and agar embedment. This, in turn, reduced the additional potential for cell damage or loss from these steps, and decreased the effort required. The method in this application note also could be applied to other types of cells and small specimens that require agar embedment.

Combining agar embedment with the rapid processing capability of the mPrep ASP-1000 enables cells to be prepared in less time and with less effort than required for manual processing. The instrument processes multiple samples simultaneously in parallel with consistent results. The walk-away convenience of unattended operation allows busy technicians to spend their valuable time on more important tasks such as microtomy, imaging and analysis.

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.
- Barcode or alphanumeric labeling of capsules reduces error potential, simplifies sample management, and enables GLP compliance.
- Automated processing in mPrep capsules provides scientists with consistent results day-in-and-day-out.

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