Apr 23, 2022

Volume Electron Microscopy Protocol for the ASP-1000

DOI
dx.doi.org/10.17504/protocols.io.q26g74mr8gwz/v1

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Protocol Citation: Erin S Stempinski, Jessica Riesterer, Steven Adamou, Claudia S Lopez 2022. Volume Electron Microscopy Protocol for the ASP-1000. **protocols.io https://dx.doi.org/10.17504/protocols.io.q26g74mr8gwz/v1**

Protocol status: Working
We use this protocol and it's working

Created: March 26, 2022

Last Modified: April 23, 2022

Protocol Integer ID: 59931

Keywords: electron microscopy, volume electron microscopy, sample preparation, ASP-1000, FIB-SEM, SBF-SEM, volume electron microscopy protocol, automated specimen processor, specimen processor, bench protocols for vem, electron beam, electron, heavy metal stain, adequate sample contrast, conductivity under the electron beam, vem, multitude of heavy metal stain

Abstract

Volume electron microscopy (vEM) requires a multitude of heavy metal stains to ensure adequate sample contrast and conductivity under the electron beam. Bench protocols for vEM can be time- and labor-intensive. The mPrep™ ASP-1000™ automated specimen processor equipped with a thermal control unit reduces the amount of time and labor required for vEM protocols.

Materials

Equipment:

Equipment	
mPrep ASP-1000 automated specimen processor equipped with Thermal Control Unit	NAME
Automated Specimen Processor	TYPE
Microscopy Innovations	BRAND
ASP1000	SKU
https://microscopyinnovations.com/	LINK

Small iron
Oven

Supplies:

- mPrep/s capsules
- mPrep/Bench silicone rack
- 12 channel reagent reservoirs, aka trays
- Pierce heat seal foil plate sheets
- Kimwipe or paper towel
- X-Pierce cross-cut plate seal sheets

Reagents:

- 0.1M sodium cacodylate, pH 7.4 5 mL 3X
- 2% osmium tetroxide (OsO_4) in 0.1M sodium cacodylate, pH 7.4 5 mL
- 2.5% w/v potassium ferricyanide in 0.1M sodium cacodylate, pH 7.4 5 mL
- high quality water, either RO or ddH20
- 1% w/v thiocarbohydrazide (TCH), aqueous 5 mL 60 °C
- 2% osmium tetroxide (OsO_4), aqueous 5 mL
- 1% w/v uranyl acetate, aqueous 5 mL
- Walton's lead stain 5 mL 60 °C
- acetone, 50%, 75%, 85% 95% 100%/200 proof (newly opened)
- EMBed 812 resin

Troubleshooting

Safety warnings

Osmium tetroxide must be used in a fume hood due to toxic vapors.

Uranyl acetate is radioactive and must be used in accordance with local guidelines.

Add the protocol to the ASP-1000

1 Add the attached protocol to the ASP-1000 protocol list. ASP1000_protocol.pdf

Prepare reagents and accessories

2 Prepare the reagents in the concentrations outlined in the materials section.

Freshly prepare day-of:

- Osmium tetroxide (OsO_4) solutions if from aqueous vials
- 1% w/v thiocarbohydrazide (TCH), aqueous - requires 1 hour to dissolve at 60°C, swirling every 10 minutes.
- EMBed812 resin

Can be prepared ahead:

- 0.1M sodium cacodylate
- 2.5% w/v potassium ferricyanide in 0.1M sodium cacodylate
- 1% w/v uranyl acetate, aqueous
- Walton's lead stain, keep at 60°C
- aqueous acetone, 50%, 75%, 85% 95%

3 Heat the small iron to the maximum temperature.

4 Heat the temperature controlled areas on the ASP-1000 to 60ºC using the thermal control unit software

Set up ASP-1000

5 Follow the attached plate map for placement of reagents in the ASP-1000 in the 12 channel reagent reservoirs. ASP1000_platemap.pdf Five to six milliliters of reagent will be placed in each channel, except for 100% acetone, which will require more.

6 Place a reservoir in the Plate 1 slot and add 0.1M sodium cacodylate in each channel used.

7 **Under the fume hood**, add reagents to a reservoir according to the plate map for Plate 2 and water for the rinse steps. Place a pierce heat seal foil plate sheet on top of the

reservoir with the blue strip facing up. Run the heated iron across the foil starting from the middle of the tray and working to the edges.

Ensure that the iron has completely sealed the foil along the edges of the reservoir. Reservoir can be taken out of the fume hood and placed in the Plate 2 slot.

Safety information

Osmium tetroxide fumes are toxic. Only work with osmium tetroxide in a fume hood. It is very important to ensure that the edges of the foil are sealed before removing the reservoir from the fume hood.

8 Ensure that Plate 3 has reached 60°C. Remove the magnetic top and place a reservoir in Plate 3. Add the fully-dissolved TCH to the appropriate area. Use water for the rinse steps. Replace the magnetic top.

9 Ensure that Plate 4 has reached 60ºC. Remove the magnetic top and place a reservoir in Plate 4. Add Walton's lead stain to the appropriate area and use water for the rinse steps. Replace the magnetic top.

10 Slide a reservoir into the Plate 6 slot. Add the uranyl acetate solution and use water for the rinse steps.

Safety information

Uranyl acetate is radioactive. Ensure you are following your institution's regulations for radioactive materials.

Prepare the sample

11

Note

Keep the sample under fluid at all times.

Place a drop of 0.1M cacodylate buffer on a cutting surface.
Dissect the sample into 1mm^3 pieces with a razor blade. Use a guillotine-like motion to minimize the damage of cells along the edge.

12 Place the mPrep/s capsules in the mPrep/Bench silicone rack. Place a few drops of 0.1M cacodylate buffer in a mPrep/s capsule. Place the samples in the mPrep/s capsule.

Note

If samples float or a specific orientation is required, place a screen inside the capsule.

Run the ASP-1000

13 Start the ASP-1000 protocol on the user interface. When prompted, attach the mPrep/s capsules to the pipette head. Remove excess fluid in the capsule with a Kimwipe or paper towel. Press "Enter" for the ASP-1000 to start the automated sample processing protocol.

14 During the end of the uranyl acetate rinse steps or the beginning of the Walton's lead stain step, prepare the final reservoir for Plate 5 with the acetone series and resin infiltration.

Note

The final 100% acetone (200 proof) step should be from a newly-opened bottle. This ensures the acetone has not absorbed water and remains 100%.

Note

Fill the channels of 100% acetone completely.

Cover the reservoir with a pierce heat foil sheet with the blue strip up. As before, iron the foil sheet beginning from the middle and working outward. Slide the reservoir into the area for Plate 5.

15 When prompted at the end of the automatic protocol, press "Enter" to raise the pipette head. Remove the capsules from the pipette head.

15.1 If polymerizing samples in the mPrep/s capsules, place the capsules in the mPrep/Bench silicone rack and add resin until desired height. Place the silicone rack in a 60°C oven.

15.2 If polymerizing samples in other molds, remove the samples from the mPrep capsule and place in prepared mold. Add resin and polymerize in an oven at 60°C.

Clean up

16 Dispose of reagents in accordance with local and institutional laws and regulations.

Remove the reagents from Plate 1 and Plate 6 and slide out the reservoirs. Reagents can removed from Plates 3 and 4 either before or after the reservoirs have been removed from the ASP-1000.

For Plate 2, place an x-pierce cross-cut plate seal sheet on top of the reservoir before removal. Place the reservoir in the fume hood before removing the reagents.

For Plate 5, slide the reservoir out of the ASP-1000. Remove any unevaporated acetone dilutions. Place the reservoir in a 60°C oven to polymerize.