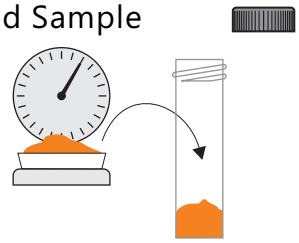
ABRAXIS® Acrylamide Plate: Sample Extraction / Centrifugation / Clean-Up
515680-ES

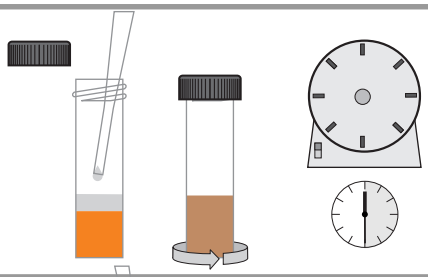
1. Weigh Homogenized Sample

Weigh 2.0 gram of homogenized sample into a 50 mL sample extraction tube.



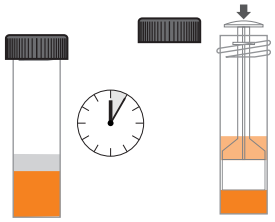
2. Extraction

Add 40 mL of de-ionized water. Vortex briefly. Place in vertical rotary mixer for 30 minutes.



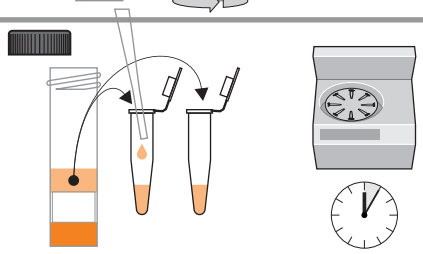
3. Filtration

Remove tube from rotary mixer and place on table top for 5 minutes (minimum) to allow for sedimentation. Using supplied plunger, push filter down the tube into the sample extract to separate solid from aqueous extract.



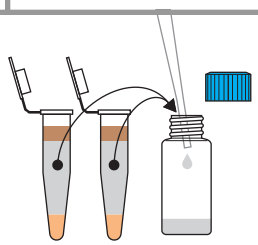
4. Centrifugation

Transfer 4 mL of aqueous extract into two separate microcentrifuge tubes (2 mL each). Centrifuge at 10,000 -15,000xg for 5 minutes at room temperature.



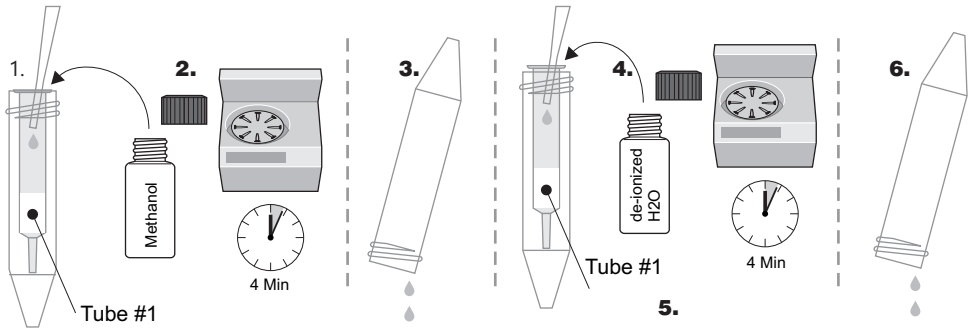
5. Removal of Extracted Acrylamide

Carefully remove 1.4 mL of supernatant from each centrifuge tube and pool sample together in a clean sample vial/tube (2.8 mL total per vial). Be sure not to remove any liquid from the top layer or to disturb the bottom layer.



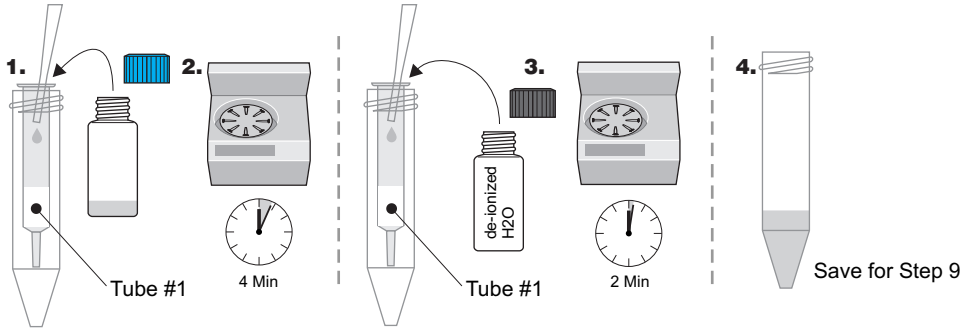
6. Condition Tube #1

1. Place Tube #1 within 15-mL centrifuge tube, labeled “Waste”. Also label sample number and save caps.
2. Condition Tube #1 with 2.0 mL 100% methanol. Centrifuge at 40 xg (490 RPM) for 4 minutes.
3. Empty contents of “Waste” centrifuge tube.
4. Rinse Tube #1 with 2.0 mL de-ionized water. Centrifuge at 40 xg (490 RPM) for 4 minutes.
5. Repeat Step 6.4.
6. Centrifuge at 40 xg (490 RPM) for 4 minutes.
7. Empty contents of “Waste” centrifuge tube.



7. Removal of Interferences

1. Place Tube #1 within clean 15-mL centrifuge tube to collect sample eluent.
2. Add 2.0 mL sample supernatant (from Acrylamide Extraction Procedure, Step 5) to Tube #1. Centrifuge at 40 xg (490 RPM) for 4 minutes.
3. Rinse Tube #1 with 1.0 mL de-ionized water. Centrifuge at 40 xg (490 RPM) for 4 minutes.
4. Save sample eluent from Tube #1 for subsequent clean-up using Tube #2.
5. Discard Tube #1.



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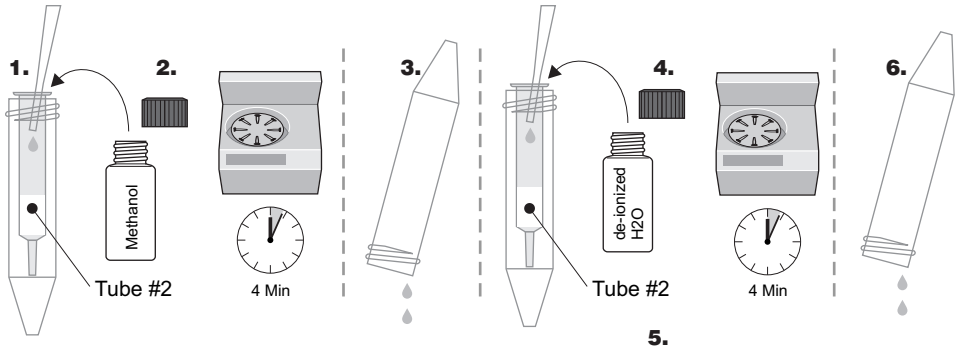
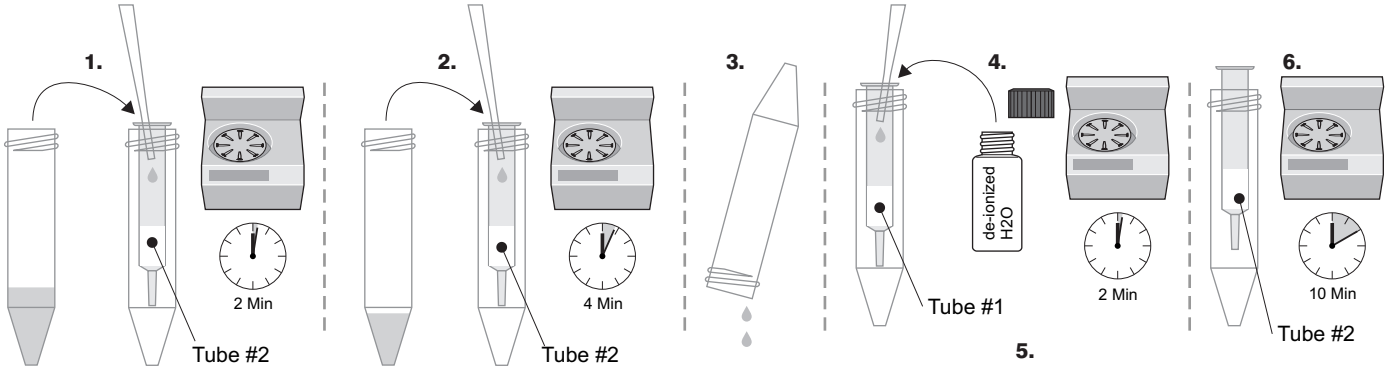
8. Condition Tube #2

1. Place Tube #2 within 15-mL centrifuge tube, labeled “Waste”.
2. Condition Tube #2 with 2.0 mL 100% methanol. Centrifuge at 40 xg (490 RPM) for 4 minutes.
3. Empty contents of “Waste” centrifuge tube.
4. Rinse Tube #2 with 2.0 mL de-ionized water. Centrifuge at 40 xg (490 RPM) for 4 minutes.
5. Empty contents of “Waste” centrifuge tube.



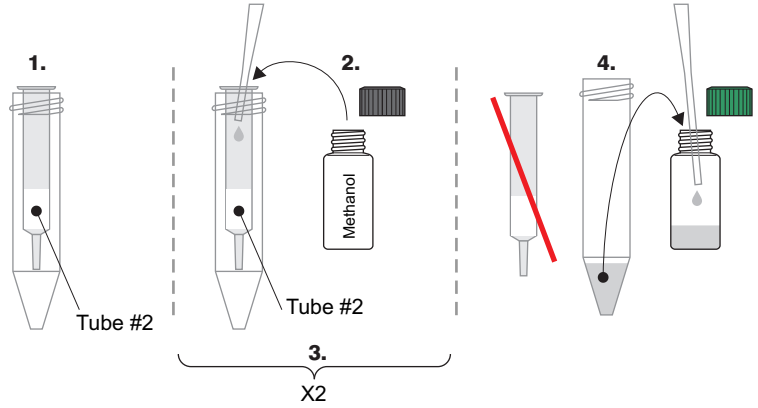
9. Concentrate Sample

1. Transfer 1.0 mL sample eluent from Tube #1 (Step 7. 4) to Tube #2. Centrifuge at 40 xg (490 RPM) for 2 minutes.
2. Transfer the rest of the sample eluent from Tube #1 (approximately 2 mL) through Tube #2. Centrifuge at 40 xg (490 RPM) for 4 minutes.
3. Empty contents of “Waste” centrifuge tube.
4. Rinse Tube #2 with 1.0 mL de-ionized water. Centrifuge at 40 xg (490 RPM) for 2 minutes.
5. Repeat Step 9.4.
6. Dry Tube #2 using centrifugation at 1,000 xg (2400 RPM) for 10 minutes.



10. Elute Sample

1. Place Tube #2 within a clean 15-mL centrifuge tube to collect sample eluent.
2. Elute extracted acrylamide from Tube #2 using 1.0 mL 60% methanol in de-ionized water.
3. Repeat Step 10.2.
4. Remove Tube #2 and transfer sample eluent (Steps 10. 2 and 10.3) to a glass sample vial.
5. Proceed to derivitization procedure.



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