

## PROPER USE OF A STEAM AUTOCLAVE

All personnel who use autoclaves at ASU must have successfully completed ASU Biosafety Training and received in-person training from their supervisor regarding the safe use and operation of autoclaves.

### MATERIAL PREPARATION

1. Ensure material is safe for autoclaving.
2. NEVER AUTOCLAVE FLAMMABLE, REACTIVE, CORROSIVE, TOXIC, or RADIOACTIVE MATERIALS.
3. Glassware must be inspected for cracks prior to autoclaving.
4. Prepare and package material suitably (refer to the chart for guidance).
5. Place items in heat-resistant secondary containers to secure and contain spills.
6. Biohazardous waste must be processed according to ASU guidelines.

### LOADING AUTOCLAVE

1. Wear Personnel Protective Equipment (PPE) including laboratory coat, eye protection, heat-insulating gloves, and closed-toe shoes. Wear an apron and face shield when handling liquids.
2. Inspect drain strainer daily. Clean when blocked.
3. Place materials in autoclave. Do not mix incompatible materials.
4. Do not overload; leave sufficient room for steam circulation. If necessary, place container on its side to maximize steam penetration and avoid entrapment of air.
5. Close and latch door firmly.

### OPERATING AUTOCLAVE

1. Close and lock door.
2. Choose appropriate cycle (e.g., gravity, liquid, or dry cycle) for the material. Consult autoclave manual for assistance in choosing the appropriate cycle (refer to chart for guidance).
3. Only designated individuals are allowed to set and/or change parameters for the autoclaves. Use indicators to determine the best treatment time.
4. Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 1 to 1.5 hours.
5. Check chamber/jacket pressure gauge for minimum pressure of 15 pounds per square inch (psi). Check temperature for 250°F (121°C) every load.
6. Do not attempt to open the door while autoclave is operating.
7. If problems with the autoclave are perceived, abort cycle and report it to the supervisor and Facilities Maintenance immediately.

### UNLOADING AUTOCLAVE

1. Wear PPE including heat-insulating gloves, eye protection, laboratory coat, and closed-toe shoes.
2. Ensure cycle has completed and both temperature and pressure have returned to a safe range.
3. Wearing PPE, stand back from the door as a precaution and carefully open door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
4. Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
5. Do not agitate containers of super-heated liquids or remove caps before unloading.
6. Wear PPE, plus an apron and face shield for liquids, to remove items from the autoclave and place them in an area which clearly indicates the items are "hot" until the items cool to room temp.
7. Shut autoclave door.
8. Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.

### AUTOCLAVE USE LOG















1. Entries must be made in the logbook each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents and/or faults.
2. Entries should include: operator's names, phone number, date, time and duration.

### MAINTENANCE AND REPAIR

1. No person shall operate the autoclave unless the autoclave is in good repair.
2. Only qualified professionals are permitted to make repairs.

### EQUIPMENT MALFUNCTION

1. If the autoclave does not operate exactly as expected, do not attempt to fix the problem.
2. Record the problem in the autoclave log book.

TYPICAL ARTICLES	Glassware	Dry Items		Liquids	Biohazard Bags	
						
PREPARATION	<b>Dirty</b>  Place in heat-resistant pan on side or inverted	<b>Clean</b>  <ol style="list-style-type: none"> <li>1. Wash</li> <li>2. Rinse</li> <li>3. Wrap*</li> <li>4. Loosen caps</li> </ol>	<b>Fabrics</b>  Wrap*    	<b>Instruments</b>  <ol style="list-style-type: none"> <li>1. Clean &amp; Dry</li> <li>2. Lay in Pan</li> <li>3. Wrap*</li> </ol> 	<ol style="list-style-type: none"> <li>1. Loosen caps or use vented closures</li> <li>2. Fill no more than 75% capacity</li> <li>3. Use Type 1 Borosilicate Glass</li> <li>4. Place in heat-resistant pan</li> </ol> 	<ol style="list-style-type: none"> <li>1. Use red/orange biohazard autoclave bags imprinted with process indicator</li> <li>2. Attach autoclave tape if indicator is not present</li> <li>3. Add 250 mL water</li> <li>4. Tie bag loosely</li> <li>5. Place in heat resistant pan</li> <li>6. Repeat cycle if indicator has not changed to show evidence of steam processing</li> </ol>
PLACEMENT IN AUTOCLAVE	<b>Detergent &amp; Water in Pan</b>  	<b>On Side Or Inverted</b>  	<b>Separated, On edge</b>  	<b>Flat in Pan</b>  	<b>Upright in Pan</b>  	<b>In Pan</b>  
TREATMENT TIME (MINUTES)	30	30	30-60	30-60	20: Test tubes 40: 1000 mL Erlenmeyer flasks	50
EXHAUST CYCLE	Slow Exhaust	Fast Exhaust & Dry	Fast Exhaust & Dry	Fast Exhaust & Dry	Slow Exhaust	Slow Exhaust
NOTES	Glassware with cracks or deep scratches may break.		*Check references for proper packaging methods.		Hot bottles may explode. Cool before moving.	Avoid puncturing bags. Dispose of waste properly.

3. Report the problem to the supervisor and Facilities Maintenance immediately.

### SPILL CLEANUP

1. Spills may occur from a boil-over, breakage of containers, or a blocked drain.
2. No operation of the autoclave is allowed until the spill is cleaned up.
3. The operator is responsible for the cleanup of spills. Contain spilled material with paper towels or other absorbent material. Use your laboratory spill kit, if necessary. Wait until the autoclave and materials have cooled to room temperature before attempting cleanup.
4. Review the manufacturer's instructions and Safety Data Sheet (SDS) to determine appropriate PPE for spill cleanup and disposal protocols.
5. Dispose of waste in accordance with regulatory requirements. If materials have been mixed, follow the cleanup and disposal protocol for the most hazardous component.
6. Cracked glassware must be disposed of properly.
7. Record the spill and cleanup procedure in the autoclave logbook.

### \* NEVER ALLOW WASTE TO ACCUMULATE IN THE LAB. NEVER LEAVE WASTE UNATTENDED.

#### INCIDENT RESPONSE

1. All incidents, including a spill or release of biohazardous materials (including recombinant / synthetic nucleic acids), must be reported to the supervisor and Biosafety & Biosecurity.
2. If an injury occurs, seek first aid. For medical assistance call dial 911.
3. If clothing is soaked, remove it and place the injury in cool water.
4. Place a sign on the unit indicating that it is not to be used until it is safe for operation.

#### REFERENCES

[EH&S Biosafety](#)  
[ASU Biosafety Manual](#)  
[CDC, Biosafety in Microbiological and Biomedical Laboratories](#)  
 DHHS: "Using the Gravity Displacement Steam Autoclave in the Biomedical Laboratory."