



## ENVIRONMENTAL HEALTH & SAFETY FACT SHEET: SPORE TESTING FOR AUTOCLAVE FUNCTION



- All autoclaves used for waste decontamination must be tested monthly for effectiveness per state regulation
- If the autoclave is only used for sterilizing of liquids or materials to be used in the lab, then the testing may be performed quarterly. Contact EH&S for permission for quarterly testing.
- Use approved spore vials (*Geobacillus stearothermophilus*) from a reputable company. EH&S recommends ProSpore® available from Thermo-Fisher #12-001-1.
- Each ProSpore® ampoule contains a spore suspension within a growth medium also containing Bromocresol Purple to function as a pH indicator. The acid production associated with growth causes a change in color from purple to or toward yellow.
- Ampoules should be purple and undamaged prior to use. Do not use after expiration date. Since ProSpore® contains live cultures, ampoules should be handled with care. ProSpore® is not intended for flash sterilization processes. This is a single use product. Sterilize all positive and expired units prior to disposal.
- Each test requires one control ampule (un-autoclaved) and one or more autoclaved ampoules
- Place one or more ProSpore® Biological Indicators in the most difficult location to sterilize, usually in the center of the load or suspended in a volume of liquid. Run cycle. Caution: After sterilization, handle ampoules with care. Contents of the ampoule are hot and under pressure. Failure to allow sufficient cooling time (10-15 minutes) may result in bursting of the ampoule.
- Place the test ampoules in a vertical position in an incubator at 55-60°C. Mark a control ampoule as such and incubate for 48 hours along with processed ampoules to ensure spore viability. Contact EH&S if you need to incubate your Prospores® in our incubator.
- Examine the ProSpore® ampoules daily during incubation. Record observations. All positive ampoules should be recorded and then disposed of immediately into a sharps container.
- Control: The control ampoule should exhibit a color change to or toward yellow and/or turbidity, which is indicative of growth. If the control ampoule does not show signs of growth, consider the test invalid.
- Test: A failed sterilization/decontamination cycle is indicated by turbidity and/or a change in color to or toward yellow. A test ampoule that retains its purple color indicates an adequate sterilization cycle. Record your results on the log sheet.
- If the test failed, perform corrective action (see below), re-process the load and run ProSpores® again. Use a different autoclave for the waste load if necessary.
- Corrective action: Check temperature charts and verify that the correct cycle was run. Verify the Prospores® are not expired and select a different lot number when retesting commences.
- Time: increase the cycle by 15 minutes and re-test. If results are positive again, increase the cycle by 15 minutes until the results are negative. The cycle time will vary depending on the size of the load.
- Density: Load the bag to 75% capacity or less, because steam cannot penetrate completely through densely packed bags.
- Steam: Add approximately 200 ml of water to dry waste loads to facilitate steam generation.
- Loading: Allow steam to better move from the top of the chamber to the bottom. Do not cover the drain. Do not let materials touch the sides or top of the autoclave.
- Record all changed parameters on the log sheet. Revise your autoclave cycle and procedures so new parameters for autoclaving waste are included and inform others who use the autoclave.
- If none of the above gives a negative result (purple autoclaved ampoule), notify EH&S and call the autoclave repair vendor.

### Expected Prospore Results:



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