

# Spore Suspension

Spore suspensions contain resistant bacterial spores for the inoculation of products used in sterilization cycle evaluation. The combined use of inoculated products, pre-sterilization microorganism count determinations (bioburden), and physical process measurements and controls should be employed to validate sterilization processes.

## DIRECTIONS FOR USE

1. Perform inoculation operations in a clean area – remote from sterility testing area.
2. Samples to be inoculated should be representative of product being sterilized.
3. Select the spore suspension of appropriate organism and population. For most purposes, inoculation of product with approximately one million ( $10^6$ ) spores provides a suitable challenge.  
NOTE: Spore suspensions are standardized on the basis of number of spores per 0.1 mL of suspension.
4. SHAKE VIAL VIGOROUSLY BEFORE EACH USE.
5. Use a sterile pipette or syringe to accurately deliver the volume of suspension to be utilized.
  - a) If syringe is used, disinfect stopper surface and pull syringe plunger halfway back. Insert needle through stopper, push plunger in, and then slowly withdraw plunger to fill syringe to desired volume.
  - b) If a pipette is used, remove stopper and insert pipette. Withdraw desired volume.
6. Deposit suspension onto product. The area to be inoculated should be the one most difficult to sterilize.
7. Allow product to dry at 20-30°C for approximately 24 hours (or until visibly dry). Some devices with small lumens may take longer to dry.
8. Package inoculated product exactly like product being sterilized and identify prominently as “Inoculated Test Samples”.
9. Distribute “Inoculated Test Samples” throughout the sterilizer load.
10. After sterilization cycle is complete, test the inoculated products as soon as possible using soybean casein digest broth and incubate at the optimal temperature for up to seven days.

**Organism...***Bacillus subtilis* ‘5230’

**Optimal Temperature...**30-35°C