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Sterilizing Polystyrene Microspheres

Procedure

Researchers are advised to optimize the use of particles in any application.

Gamma Irradiation

A dose of 0.030 megarad/hour for 24 hours (0.72 megarad) is sufficient to control most bacteria and contaminating molds; however, a few yeast cells may survive beyond 100 hours (3.0 megarads). Particles irradiated after packaging at >2 megarads (67 hours) gives excellent results. In some cases, the radiation will cause discoloration of the packaging or certain soluble materials, but the particles seem to be unaffected. This process, if available, is quite effective for sterilizing the particles.

Heat Treatment of Aqueous Suspensions

This applies to aqueous suspensions in high density polyethylene bottles and closures.

1. Preheat an appropriately sized, calibrated oven to 80˚C ± 4˚C.
2. Place bottles into preheated 80˚C oven. Each bottle may touch another bottle, but may not touch the walls or top of the oven. Only the bottom of the bottles may come in contact with the oven surface.
3. Allow the material to remain in the oven for 2 hours ± 10 minutes.
4. Remove from 80˚C oven and incubate in a preheated 40˚C ± 4˚C calibrated oven for 20 hours ± 4 hours. The same oven may be used for this temperature cycling if the temperature is reduced from 80˚C to 40˚C in one hour.
5. Remove from 40˚C oven and incubate in 80˚C ± 4˚C calibrated oven for 2 hours ± 10 minutes. The same oven may be used for this temperature cycling if the temperature is increased from 40˚C to 80˚C in one hour.
6. Remove from 80˚C oven and incubate in a preheated 40˚C ± 4˚C calibrated oven for 20 hours ± 4 hours. The same oven may be used for this temperature cycling if the temperature is reduced from 80˚C to 40˚C in one hour.
7. Remove from 40˚C oven and incubate in 80˚C ± 4˚C calibrated oven for 2 hours ± 10 minutes. The same oven may be used for this temperature cycling if the temperature is increased from 40˚C to 80˚C in one hour.
8. In total, the product should be exposed to three cycles of 80˚C and two cycles of 40˚C.

Sterilization by Rinsing with 70% Ethanol or 70% Isopropyl Alcohol

Notes:

- This procedure should only be used with undyed, uncolored polystyrene microspheres. Exposure of colored beads to Ethanol or Isopropyl Alcohol will leach out the dye.
- Particles that are 0.5µm or larger should be used as they will be able to form a pellet by centrifugation. For particles smaller than 0.5µm, a hollow fiber filter or dialysis tubing must be used to concentrate the particles.
- Size standard particles should not be exposed to Ethanol or Isopropyl Alcohol because it will temporarily swell the particles. They will return to their approximate size once resuspended in water, but their accuracy as size standards may be compromised. Use an alternate method for sterilization.

1. Mix the bottle of particles by inverting the bottle several times to achieve an even distribution of the particles before taking an aliquot.
2. Place an aliquot of the bead suspension into a centrifuge tube. Centrifuge the particles to form a visible, white pellet at the bottom of the tube following the instructions on the next page.
3. Remove the water supernatant and replace with an equivalent volume of 70% Ethanol or 70% Isopropyl Alcohol.

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4. Vortex briefly to mix, then centrifuge down to pellet and remove the Ethanol or Isopropyl Alcohol, replacing with fresh 70% Ethanol or 70% Isopropyl Alcohol. Repeat this two times.
5. After the last 70% Ethanol or 70% Isopropyl Alcohol rinse, centrifuge to form a pellet and resuspend in an equivalent amount of sterile DI water or the desired sterile aqueous buffer. Vortex briefly to mix.
6. Centrifuge to form a pellet, remove supernatant, and replace with fresh sterile DI water or sterile aqueous buffer.
7. Repeat the sterile DI water or sterile buffer wash two more times to remove all traces of Ethanol or Isopropyl Alcohol before using the particles. Particles should now be ready to use.

Centrifugation

Washing particles may be done via centrifugation. This procedure must be performed carefully. Excess centrifugation will result in resuspension difficulties. For the purposes of pelletizing, it is important to understand the settling velocities of particles.

For spherical particles, settling velocity can be calculated using Stokes' Law.

$$V = \frac{2ga^2 (\rho_1 - \rho_2)}{9n}$$

V	=	Velocity in cm/sec
g	=	g force in cm/sec^2
ρ_1	=	density of particle in g/cm^3
ρ_2	=	density of suspending media in g/cm^3
n	=	coefficient of viscosity in poises (g/cm-sec)
a	=	radius of spherical particle in cm

For calculating the settling velocity of polystyrene microspheres at 1G in 20˚C water, Stokes' Law can be expressed in the following formula, where d = diameter in microns, ρ_1 = 1.05 g/cm^3, ρ_2 = 1.00 g/cm^3, and n = 1.002 cp.

$$V = 2.77 \times 10^{-6} d^2$$

To estimate appropriate times for centrifugation, settling velocity is multiplied by the G forces generated by the centrifuge. The resultant velocity is then compared to the height of the centrifuge tube.

For example: A 1.0µm particle placed in a microcentrifuge generating 10,000 G will settle at a velocity of 2.77 x 10^2 cm/sec. Pelletizing the particle in a 4cm high tube would require a 144 second (minimum) centrifuge run. The actual time required to form an acceptable pellet could possibly be 50% or longer. These calculations are intended to be used as guidelines to assist in determining centrifugation time. Different size particles yield dramatically different settling velocities. A 10.0µm particle could settle in 2 seconds under the aforementioned conditions, whereas a 0.01µm particle could take at least 4 hours to settle. Brownian motion and particle concentration also affect settling rates.

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