



Decontaminating Microspheres

DESCRIPTION

Though many of our microsphere suspensions contain an antimicrobial agent, certain applications require sterile or near-sterile preparations. In these instances, suspensions may be treated with radiation, heat, or chemicals for decontamination or true sterilization.

PROCEDURE

Though most of our microspheres are comprised of materials that withstand typical decontamination processes (PS, PMMA, silica, magnetic polymer), it is important to note that some methods may be more fitting than others depending on the material, required level of decontamination, and the intended application. Microsphere modifications (dyeing, coating) and packaging should also be considered to ensure compatibility. Decontamination processes should be optimized, validated, and monitored to ensure efficacy.

Irradiation

Irradiation has grown in its use for sterilizing food, consumer products, and medical supplies and devices. Gamma irradiation is characterized by deep penetration and low dose / longer irradiation times (hours, days), while electron beam (e-beam) irradiation features shallow penetration and high dose / short irradiation times (minutes). Both are highly effective methods of sterilization, and suitable for a variety of materials. Irradiation is typically conducted by a specialty service provider who additionally assists with sterilization dose selection, product dose mapping, and related validations.

Heat Treatment of Aqueous Suspensions

This applies to aqueous suspensions in high density polyethylene bottles and closures. Bottle closures should be loosened to avoid deformation of the bottle.

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 is intended for undyed and uncoated microspheres and should be tested prior to use with other bead types to ensure compatibility.
- Particles that are 0.5µm or larger in diameter 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 may 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.
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.

Microsphere Handling

Additional information on conducting washes / separations is provided in *TechNote 203, Washing Microspheres*.

Separations

Particle washing is typically accomplished via centrifugation (spheres ≥0.5µm). This procedure must be performed carefully. Excess centrifugation will result in resuspending difficulties. Spheres that are <0.5µm, we suggest the use of our Vivaspin® Ultrafiltration devices. See *PDS AA022* for details. General reference ranges are provided on the next page. These are intended to serve only as starting points, and should be optimized or adapted as appropriate. *TechNote 206, Equations*, provides an additional reference table and equation for calculating settling velocities.

CENTRIFUGATION CHART

BEAD TYPE	DIAMETER RANGE	RELATIVE CENTRIFUGAL FORCE RANGE (×G)	SPEED RANGE (RPM)
polymer	> 0.5µm	6500-14000	8925-13100
	> 1.0µm	3000-5500	6060-8210
	> 5µm	1300-3000	3990-6060
silica	> 0.5µm	3000-5500	6060-8210
	> 1.0µm	1300-3000	3990-6060
	> 5.0µm	750-1300	3030-3990
protein/Ab-coated	> 0.5µm	8000-11000	9900-11610
	> 1.0µm	5500-8000	8210-9900
	> 5.0µm	2000-5500	4950-8210

Note: Forces are based upon a 5 minute centrifugation time and a 7.3cm rotation radius (benchtop microcentrifuge).

STORAGE AND STABILITY

Aqueous microsphere suspensions should be stored at 2-8°C to deter microbial growth. Freezing of particles may result in irreversible aggregation and loss of binding activity. Inclusion of an antimicrobial agent may be considered, e.g. sodium azide or ProClin®.

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