3. Allow all components, except the burst disks, to soak for at least 1 h to sterilize. Sterilize the burst disks by soaking for 5 min. After sterilization, transfer all components to a layer of autoclaved wipes to dry.

3.2.1. Preparation of Microcarriers

The following method is modified slightly from that of Heiser ***(5)*** and was originally developed by Sanford et al. ***(4)***. It allows the preparation of 30 mg of particles, which would be adequate for 50 individual bombardments. We usually prepare 30 mg, since the particles can be stored for only about 3 wk at 4°C, or 2 wk at room temperature. For ease of handling, when 60 mg are needed, we prepare two tubes with 30 mg each.

1. In a 1.5-mL microcentrifuge tube, vigorously vortex 30 mg of particles in 0.5 mL freshly prepared 70% EtOH for 5 min.
2. Incubate particles at room temperature for 15 min.
3. Microcentrifuge (15,000*g*) for 5 s and decant.
4. Wash particles three times as follows:
   a. Add 0.5 mL sterile water.
   b. Vortex for 1 min.
   c. Allow particles to settle for 1 min.
   d. Microcentrifuge for 5 s and decant.
5. Add sterile 50% glycerol to bring the particle concentration to 60 mg/mL.
6. While vigorously vortexing, distribute 40-µL aliquots of particles into 1.5-mL microcentrifuge tubes.

3.2.2. Coating DNA on Microcarriers

1. Add 1 and 3 µL (1 mg/mL) $CsCl_2$-purified plasmid encoding the bialaphos-resistance gene and the gene of interest, respectively, to a 40 µL aliquot of particles (*see* **Note 2**). We usually bombard with four different constructs in a given experiment, which results in coating four aliquots of particles at a time.
2. Place tubes on a table mixer (e.g., Eppendorf Model 5432) and mix for 5 min.
3. While vortexing vigorously, add 40 µL $CaCl_2$ (2.5*M*) and then 16 µL spermidine (0.1*M*).
4. Continue vigorous vortexing for 3 min.
5. Allow particles to settle (for at least 1 min) while subsequent aliquots are being processed.
6. Microcentrifuge for 4–5 s.
7. Draw off supernatant with a pipet and discard.
8. Without disturbing pellet, add 112 µL freshly prepared 70% EtOH.
9. Draw off supernatant with a pipet and discard.
10. Again without disturbing pellet, add 112 µL 100% EtOH.
11. Draw off supernatant with a pipet and discard.
12. Suspend particles in 39 µL of 100% EtOH (a few microliters will be lost to evaporation and 36 µL are needed) by mixing with the pipet tip and then vigorously pipeting up and down.