MODEL PDS-1000/He BIOLISTIC® PARTICLE DELIVERY SYSTEM

CONDITIONS OF USE

The Biolistic PDS-1000/He apparatus is for research purposes only. Research purposes shall not include transfers of materials produced by the apparatus to third parties for consideration or research programs that are funded by or for third party commercial (for profit) entities. In the event that anyone wishes to use the Biolistic PDS-1000/He apparatus for commercial purposes, such person may contact W.R. Grace and Co.-Conn. for rights in the mammalian field and E.I. Du Pont de Nemours and Co. for rights in all other fields. No license can be granted for trees, shrubs, ground cover used for indoor and outdoor landscaping, ornamental flowers, house plants, vines and turf grasses. For the purpose of the agreement “use for commercial purposes” shall include the production, use, or transfer for consideration of apparatus, process, or product for performing the Biolistic process.

Biolistic® -PDS-1000/He Particle Delivery System

The following material with regards to the Biolistic Particle Delivery System can be found in the Biolistic® -PDS-1000/He Particle Delivery System supplied by Bio-Rad@http://www.biorad.com.

Biolistic particle delivery is a method of transformation that uses helium pressure to introduce DNA-coated microcarriers into cells. Microprojectile bombardment can transform such diverse targets as bacterial, fungal, insect, plant, and animal cell and intracellular organelles. Particle delivery is a convenient method for transforming intact cells in culture since minimal pre- or post-bombardment manipulation is necessary. In addition, this technique is much easier and faster to perform than the tedious task of micro-injection. Both stable and transient transformation are possible with the Biolistic particle delivery system.

The Biolistic PDS-1000/He system uses high pressure helium, released by a rupture disk, and partial vacuum to propel a macrocarrier sheet loaded with millions of microscopic tungsten or gold microcarriers toward target cells at high velocity. The microcarriers are coated with DNA or other biological material for transformation. The macrocarrier is halted after a short distance by a stopping screen. The DNA-coated microcarriers continue traveling toward the target to penetrate and transform the cells.
The launch velocity of microcarriers for each bombardment is dependent upon the helium pressure (rupture disk selection), the amount of vacuum in the bombardment chamber, the distance from the rupture disk to the macrocarrier, the macrocarrier travel distance to the stopping screen, and the distance between the stopping screen and target cells.

Consumable Preparation

1) Macrocarriers

Pre-assemble and pre-sterilize the macrocarrier set in a macrocarrier holder prior to performing sample cell/tissue bombardments. Place the macrocarrier into the macrocarrier holder using the seating tool. The edge of the macrocarrier should be securely inserted under the lip of the macrocarrier holder. The macrocarriers holders, with macrocarriers already in place, should be sterilized by autoclaving.

2) Rupture disks

Transfer selected rupture disks to individual Petri dishes for easier handling. Sterilize rupture disks by briefly dipping them in 70% isopropanol just prior to insertion in the Retaining Cap. Do not soak for more than a few seconds. Extensive soaking may delaminate the disks, resulting in premature rupture. All disks, with the exception of those rated at 450, 650, and 1,100psi. are laminated. Autoclaving is not recommended because of potential delamination.

3) Stopping screens

Transfer selected stopping screens to individual Petri dishes for easier handling. sterilization by autoclaving is recommended. Alternatively, these parts can be sterilized by soaking in 70% ethanol, followed by drying in a sterile environment.

4) Microcarriers

The following procedure prepares tungsten or gold microcarriers for 120 bombardments using 500µg of the microcarrier per bombardment, based on the method of Sanford, *et al.[Methods in Enzymology*, **217**. 482-509 (1993)].
Weigh out 30 mg of microparticles into a 1.5 ml microfuge tube.
Add 1 ml of 70% ethanol (v/v).
Vortex vigorously for 3-5 minutes (a platform vortexer is useful).
Allow the particles to soak in 70% ethanol for 15 minutes.
Pellet the microparticles by spinning for 5 seconds in a microfuge.
Remove and discard the supernatant.
Repeat the following wash steps three times:

- Add 1 ml of sterile water.
- Vortex vigorously for 1 minute.
- Allow the particles to settle for 1 minute.
- Pellet the microparticles by briefly spinning in a microfuge.
- Remove the liquid and discard.

After the third wash, add 500µl sterile 50% glygerol to bring the microparticle concentration to 60 mg/ml (assume no loss during preparation).
The microparticles can be stored at room temperature for up to two weeks. Tungsten aliquots should be stored at –20 C. to prevent oxidation. Gold aliquots can be stored at 4 C or room temperature.
Store dry tungsten and gold microcarriers in a dry, non-oxidizing environment to minimize agglomeration.

Coating Washed Microcarriers with DNA

The following procedure is sufficient for six bombardments; if fewer bombardments are needed, adjust the quantities accordingly.

Vortex the microcarriers prepared in 50% glycerol (30mg/ml) for 5 minutes on a platform vortexer to resuspend and disrupt agglomerated particles.
When removing aliquots of microcarriers, it is important to continuously vortex the tube containing the microcarriers to maximize uniform sampling. When pipetting aliquots, hold the microcentrifuge tube firmly at the top while continually vortexing the base of the tube.
Remove 50µl (3 mg) of the microcarriers to a 1.5 ml centrifuge tube.
Continuous agitation of the microcarriers is needed for uniform DNA precipitation onto microcarriers. For added convenience and/or multiple samples, use a platform attachment on your vortex mixer for holding microcentrifuge tubes.
While vortexing vigorously, add in order:

5 µl DNA (1µg/µl)
50µl 2.5 M $CaCl_2$
20µl 0,1 M spermidine (free base, tissue culture grade)
Continue vortexing for 2-3 minutes.
Allow the microcarriers to settle for 1 minute.
Pellet microcarriers by spinning for 2 seconds in a microfuge.
Remove the liquid and discard.
Add 140µl of 70% ethanol (HPLC of spectrophotometric grade).
Remove the liquid and discard.
Add 140µl of 100% ethanol.
Remove the liquid and discard.
Add 48 µl of 100% ethanol.
Gently resuspend the pellet by tapping the side of the tube several times, and then by vortexing at low speed for 2-3 seconds.

Performing a Bombardment

Before the Bombardment

1) Select/adjust bombardment parameters for gap distance between rupture disk retaining cap and microcarrier assembly. Placement of stopping screen support in proper position inside fixed nest of microcarier launch assembly.
2) Check helium supply (200 psi in excess of desired rupture pressure).
3) Clean/sterilize:
   Equipment: rupture disk retaining cap, microcarrier launch assembly
   Consumables: macrocarriers/macrocarrier holders.
4) Wash microcarriers and resuspend in 50% glycerol.
5) Coat microcarriers with DNA and load onto sterile macrocarrier/macrocarrier holder the day of the experiment

Firing the Device

1) Plug in power cord form main unit to electrical outlet.
2) Power ON.
3) Sterilize chamber walls with 70% ethanol.
4) Load sterile rupture disk into sterile retaining cap.
5) Secure retaining cap to end of gas acceleration tube (inside, top of bombardment chamber) and tighten with torque wrench.
6) Load macrocarrier and stopping screen into microcarrier launch assembly.
7) Place microcarrier launch assembly and target cell in chamber and close door.
8) Evacuate chamber, hold vacuum at desired level (minimum 5 inches of mercury).
9) Bombard sample: Fire button continuously depressed until rupture disk bursts and helium pressure drops to zero
10) Release Fire button.

After the Bombardment

1) Release vacuum from chamber.
2) Target cells removed from chamber.
3) Unload macrocarrier and stopping screen from macrocarrier launch assembly.
4) Unload spent rupture disk.
5) Remove helium pressure from the system (after all experiments completed for the day).