

Section 4

Operation of the PDS-1000/He Instrument

4.1 Preparation of System Components Prior to Bombardment

Instrument Preparation

1. Verify that helium tank has 200 psi in excess of desired rupture disk pressure for bombardment.
2. Set gap distance between rupture disk retaining cap and microcarrier launch assembly.

When the rupture disk retaining cap is in place, insert the fully assembled microcarrier launch assembly (with cover lid) inside the bombardment chamber on the highest possible bombardment chamber wall slot (Figure 4.1). The set screw in the white plastic shelf should face outward. Release the set screw on the front of the microcarrier launch assembly with the smaller of the two hex key wrenches provided (Figure 2.4).

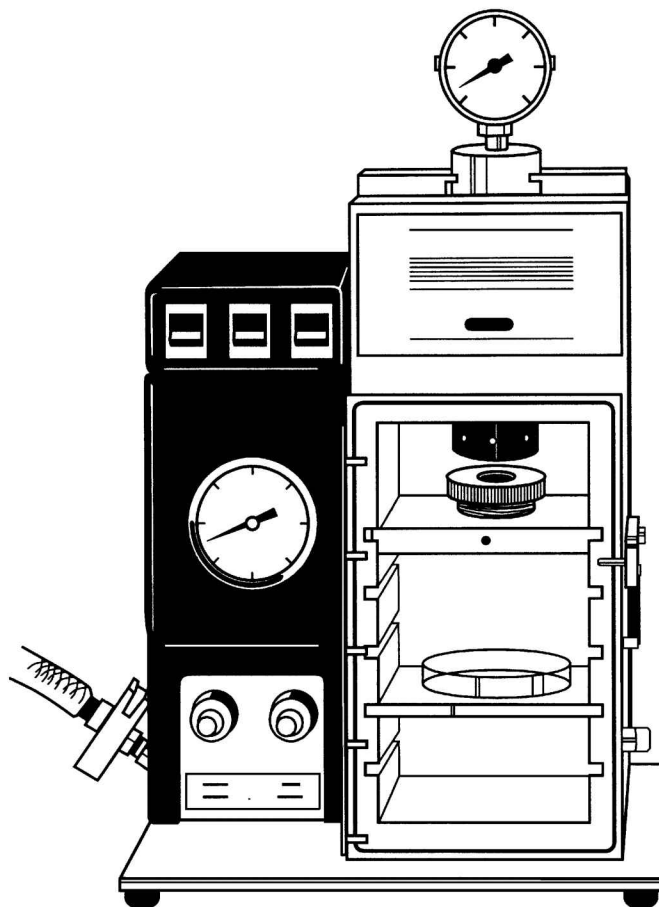


Fig. 4.1. Microcarrier launch assembly and target shelf inside bombardment chamber.

Three hexagonal gap adjustment tools of 1/8", 1/4", and 3/8" have been provided to reproducibly set the gap distance (Figure 2.4). A 1/4" distance between the rupture disk retaining cap and the macrocarrier cover lid is recommended for initial optimization bombardments.

While holding the hexagonal gap adjustment tool against the bottom of the rupture disk retaining cap, turn the adjustable nest until the macrocarrier cover lid touches the gap adjustment tool. The position of the adjustable nest is fixed by tightening the set screw in the white plastic shelf with the hex key wrench.

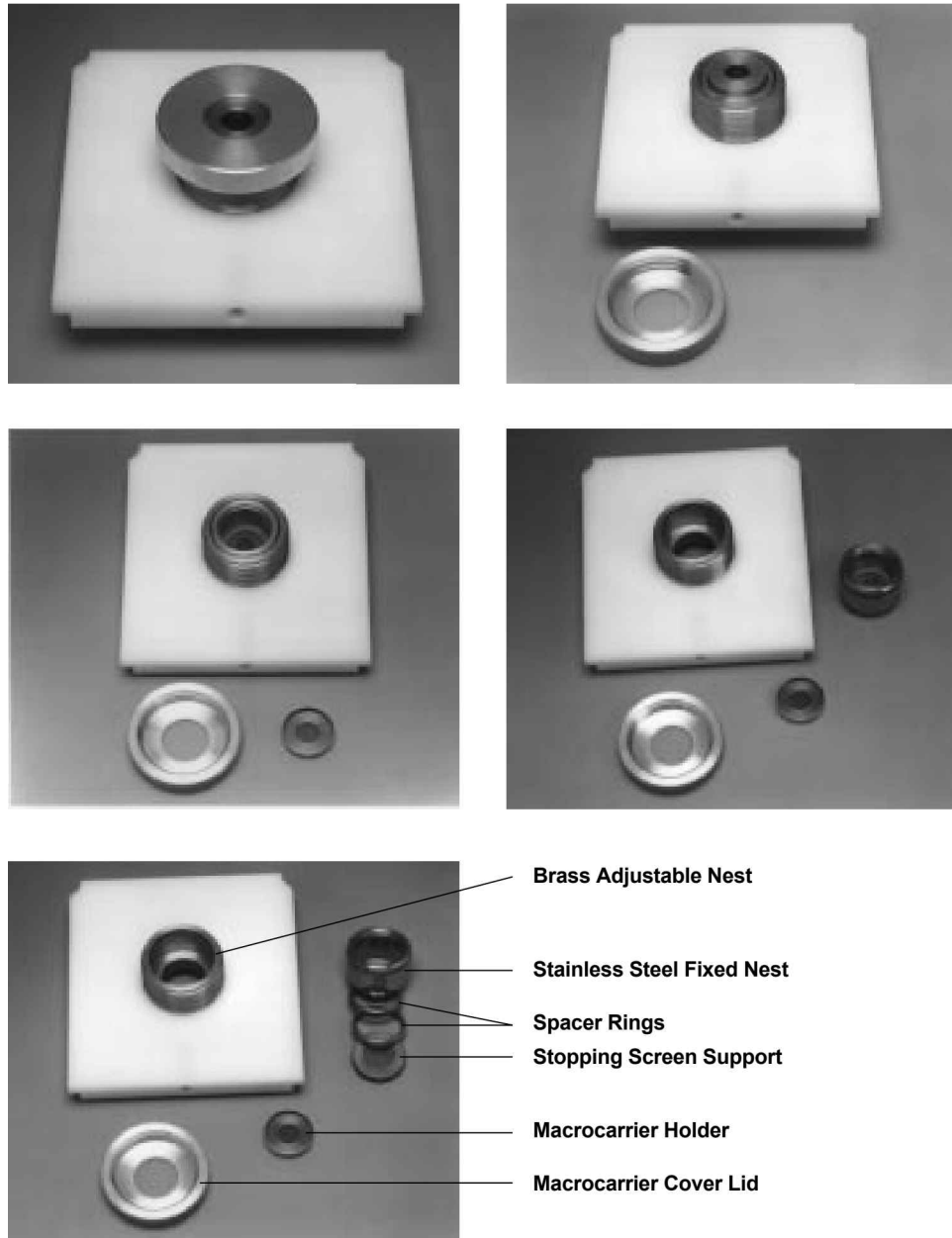


Fig. 4.2. Microcarrier Launch Assembly adjustment.

3. Prepare the Rupture Disk Retaining Cap. After setting the gap between cap and microcarrier launch assembly, wrap the Rupture Disk Retaining Cap in aluminum foil and sterilize by autoclaving.

4. Prepare the Microcarrier Launch Assembly.

The effect of the gas shock wave on the microcarrier velocities is determined in part by the gap between the rupture disk and the macrocarrier.

The macrocarrier flight distance can be adjusted by varying the positions of the stopping screen support and the spacer rings inside the fixed nest (Figure 4.2). The Stopping Screen Support is placed in the middle position at the factory. This is the recommended position when initially optimizing bombardment parameters.

To make this adjustment, remove the microcarrier launch assembly from the PDS-1000/He unit. Unscrew and remove the macrocarrier cover lid. Disassemble the components of the microcarrier launch assembly by placing the macrocarrier insertion tool into the bottom of the assembly and pushing up. This releases the stainless steel fixed nest from the brass adjustable nest. The two spacer rings and the stopping screen support will fall out from within the fixed nest.

To change the factory-set position to the position for the minimum macrocarrier travel distance (6 mm), invert the fixed nest and insert the stopping screen support inside the fixed nest so that the conical side of the stopping screen support faces down in the final orientation; then insert the two spacer rings (5 mm thickness, each). With the fixed nest still inverted, place the macrocarrier launch assembly over the fixed nest and seat the fixed nest within the brass adjustable nest.

If greater macrocarrier travel is desired, rearrange the spacer rings and stopping screen support accordingly. The macrocarrier travel distance can be increased in two 5 mm steps, to a maximum of 16 mm.

Sterilize the microcarrier launch assembly by wrapping in aluminum foil and autoclaving. Alternatively, this assembly can be sterilized by wiping with 70% ethanol, followed by drying in a sterile environment.

5. Target Shelf

Sterilize the target shelf by wiping with 70% ethanol, followed by drying in a sterile environment just prior to use. This part may not be autoclaved.

Consumable Preparation

Several consumables are available for use with the PDS-1000/He system (Figure 4.3).

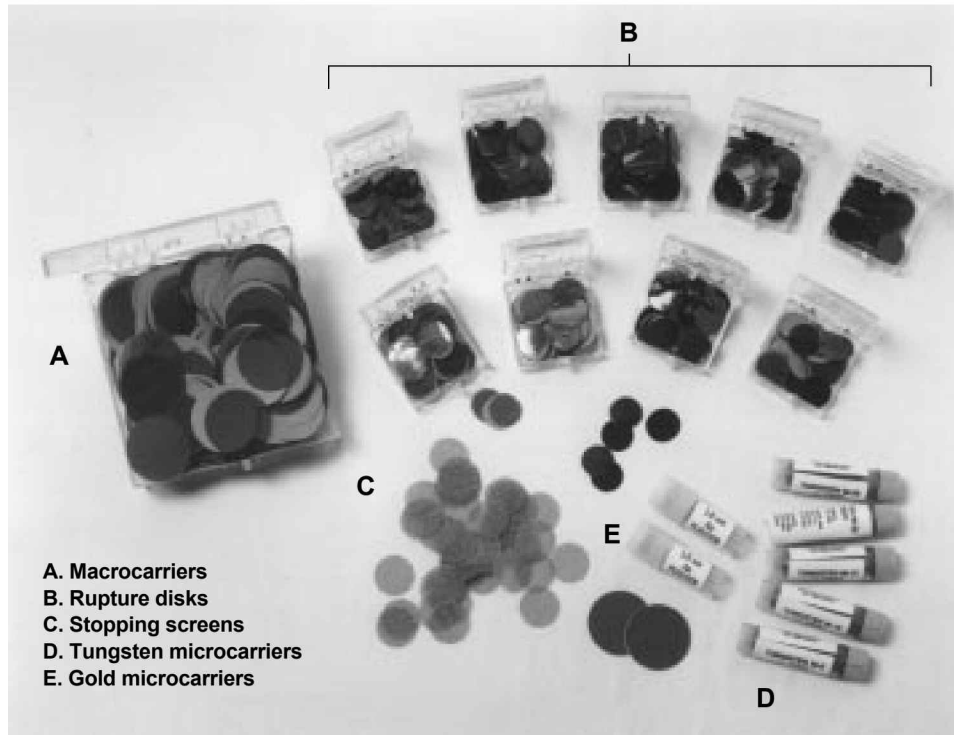


Fig. 4.3. Consumables for the PDS-1000/He instrument. The 0.6 micron gold is not pictured.

1. Macrocarriers

Pre-assemble and pre-sterilize the macrocarrier set in a macrocarrier holder prior to performing sample cell/tissue bombardments. The Disk-Vac (catalog number 165-2323) provides ease in handling rupture disks and macrocarriers.

Macrocarriers are shipped in quantities of 500/box, with paper linings between disks. Transfer selected macrocarriers to individual Petri dishes for easier handling. Remove the paper lining from between the macrocarriers. Place the macrocarrier into the macrocarrier holder using the seating tool (Figure 4.4). The edge of the macrocarrier should be securely inserted under the lip of the macrocarrier holder. The macrocarrier holders, with macrocarriers already in place, should be sterilized by autoclaving.

2. Rupture disks

Rupture disks (Figure 4.3) are packaged and shipped in quantities of 100/box. 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 1,100 psi, are laminated. Autoclaving is not recommended because of potential delamination.

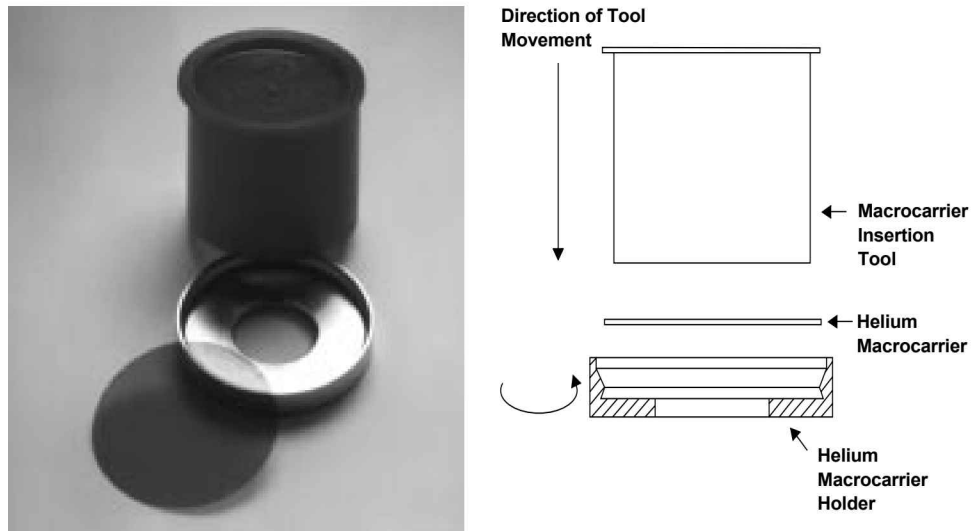


Fig. 4.4A and 4.4B. Insertion of macrocarrier into macrocarrier holder with plastic insertion tool.

3. Stopping screens

Transfer selected stopping screens (Figure 4.3) 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 500 μg 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 500 μl sterile 50% glycerol 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 $-20\text{ }^{\circ}\text{C}$ to prevent oxidation. Gold aliquots can be stored at $4\text{ }^{\circ}\text{C}$ 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 (30 mg/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 50 μ l (3 mg) of microcarriers to a 1.5 ml microcentrifuge 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 μ l DNA (1 μ g/ μ l)
- 50 μ l 2.5 M CaCl_2
- 20 μ l 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 140 μ l of 70% ethanol (HPLC or spectrophotometric grade).

Remove the liquid and discard.

Add 140 μ l of 100% ethanol.

Remove the liquid and discard.

Add 48 μ l 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.

4.2 Performing a Bombardment

Quick Guide (This summary is repeated in Section 8.4 as a tear-out sheet.)

Before the Bombardment

1. Select/adjust bombardment parameters for gap distance between rupture disk retaining cap and microcarrier launch assembly. Placement of stopping screen support in proper position inside fixed nest of microcarrier 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 experiment

Firing the Device

1. Plug in power cord from 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 cells 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 gauge 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 microcarrier launch assembly.
4. Unload spent rupture disk.
5. Remove helium pressure from the system (after all experiments completed for the day).

Detailed Operation Procedure

Note: We recommend that the first bombardment each day be a “dry run” with no target cells or microcarriers to ensure that the system is set up properly and the gas pathway is filled with helium, not air.

1. Power On

With the unit plugged in to the appropriate electrical outlet or voltage converter, turn on the unit by pressing the ON switch. This is the left-most red control panel switch (Figure 2.9).

2. Helium Pressure

Confirm that the helium tank pressure regulator is set to 200 psi over the selected rupture disk burst pressure (*e.g.*, set the regulator to 1,100 psi when working with a 900 psi rupture disk).

3. Pressurizing the System with Helium

Make certain that the helium pressure regulator is properly installed on the helium tank (see Set-up).

Close the helium pressure regulator by turning the regulator adjustment screw counter-clockwise until the adjusting spring pressure is released.

Release helium into the pressure regulator by carefully and slowly opening the cylinder valve on the helium tank. The cylinder pressure in the tank is indicated on the high pressure gauge (the gauge closest to cylinder).

Set the desired helium delivery pressure for the rupture disk you are using (measured on gauge on the outside, farthest from cylinder) by turning the regulator adjustment handle clockwise. The pressure should be set to 200 psi above the rupture disk rating.

4. Coating microcarriers with DNA

The day of the scheduled bombardment, coat the microcarriers with DNA. To obtain the best results, use the DNA-coated microcarriers as soon as possible.

5. Loading DNA-coated microcarriers onto a macrocarrier/macrocarrier holder

Each macrocarrier is placed inside a macrocarrier holder and sterilized, as described above. Prior to the application of the DNA-coated microcarriers onto a macrocarrier, prepare a small desiccating chamber for each macrocarrier/macrocarrier holder and place away from excessive vibration.

A small desiccating chamber consists of a sterile 60 mm tissue culture Petri dish (with lid) containing CaCl_2 as desiccant in the base of the dish (Figures 4.5 and 4.6). The desiccant is covered with a small piece of filter paper to provide a stable platform for the macrocarrier/macrocarrier holder. The sterile macrocarrier/macrocarrier holder is placed atop the filter paper, with the macrocarrier facing up and the stainless steel holder touching the filter paper.

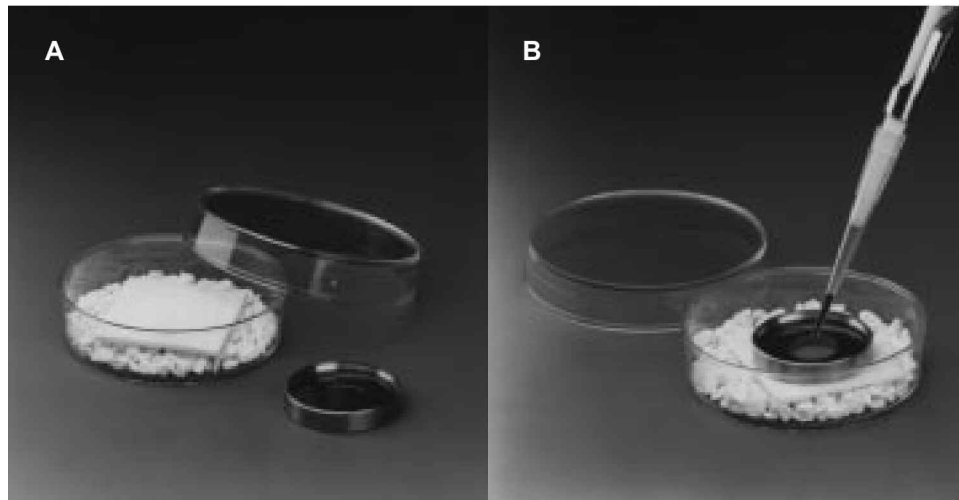


Fig. 4.5A and 4.5B. Loading DNA-coated microcarriers onto a macrocarrier/macrocarrier holder, positioned in small desiccating chamber.

This environment permits rapid access to each macrocarrier and provides a low humidity environment for the ethanol to uniformly evaporate from the microcarriers. This low humidity, along with a minimum of vibration during evaporation, minimizes microcarrier agglomeration.

For each macrocarrier, remove 6 μl aliquots (approximately 500 μg) of microcarriers and spread evenly over the central 1 cm of the macrocarrier using a pipette tip. Pipet from a continuously vortexed tube and rapidly apply suspended microcarriers to the macrocarrier, as microcarriers quickly settle out from the ethanol solution in the tube or even in the pipette tip.

Immediately cover the culture dish after application of the microcarrier suspension to the macrocarrier. The ethanol should evaporate within 10 minutes to leave the DNA-coated microcarriers adhering to the macrocarrier. The loaded macrocarriers should be used within 2 hours.

6. Cleaning chamber walls

Clean the chamber of the PDS-1000/He as desired with 70% ethanol. Allow time for drying. Do not autoclave or flame sterilize the PDS-1000/He unit.

7. Loading the rupture disk

Unscrew the rupture disk retaining cap from the gas acceleration tube from within the bombardment chamber (Figure 4.6) or unwrap cap from sterile wrapping.

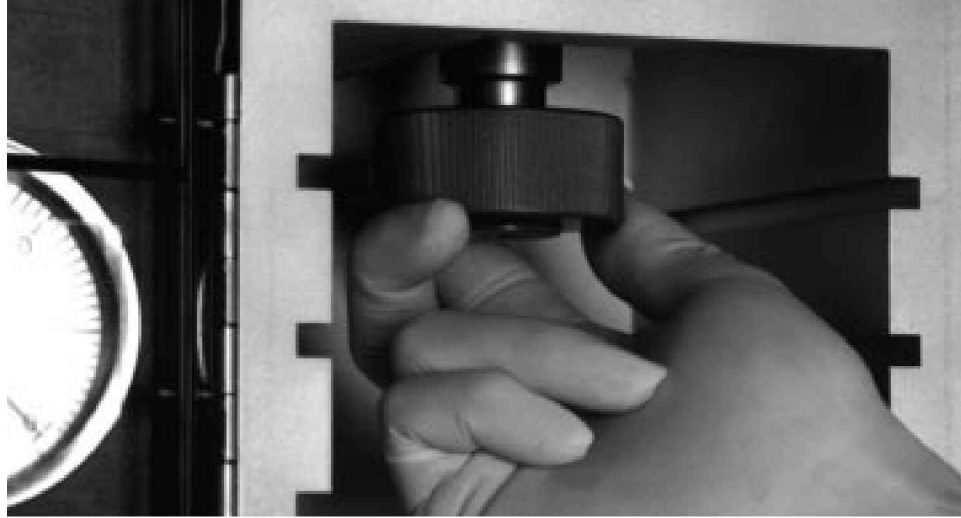


Fig. 4.6. Removal/mounting of rupture disk retaining cap onto end of gas acceleration tube inside bombardment chamber.

Select rupture disk of desired burst pressure. Handle all rupture disks with sterile forceps or Disk-Vac (catalog number 165-2323). Grease, fingerprints, or even powder from plastic gloves on the rupture disk may prevent a tight seal from forming within the retaining cap.



Fig. 4.7. Rupture disk insertion into recess of retaining cap.

Immediately before placing a rupture disk in the retaining cap, briefly wet the rupture disk in isopropanol (do not soak the disk for an extended period of time or the disk may delaminate). Loading the rupture disk while wet forms a liquid gasket when the cap is screwed onto the gas acceleration tube, and thereby reduces the failure rate of the rupture disk. Place the rupture disk in the recess of the rupture disk retaining cap (Figure 4.7).

Screw the rupture disk retaining cap onto the gas acceleration tube using a left-to-right motion. Never tighten the rupture disk retaining cap without a rupture disk in place or scratching and deformation of the two metal surfaces will occur and cause helium to leak when a rupture disk is pressurized.

The retaining cap is tightened to a torque of 60 inch/pounds with the retaining cap torque wrench. To use the torque wrench, insert the short end of the metal rod into an accessible hole in the retaining cap. Push the long end of the rod to the right only until it touches the inner surface of the black tube (Figure 4.8). If the retaining cap is not tightened sufficiently, the rupture disk may slip out of place as the gas acceleration tube fills with helium. Test fire once to fill gas tubing with helium.



Fig. 4.8. Proper torque applied to retaining cap with torque wrench.

8. Microcarrier Launch Assembly

Unscrew the macrocarrier cover lid from the assembly. Place a sterile stopping screen on the stopping screen support (Figure 4.9). **Note:** Never operate the PDS-1000/He instrument without a stopping screen in place. The target sample will be destroyed from the uninterrupted acceleration of the macrocarrier by the helium shock wave.

Install the macrocarrier/macrocarrier holder on the top rim of the fixed nest (Figure 4.10). The dried microcarriers should be facing down, toward the stopping screen. Replace the macrocarrier cover lid on the assembly and turn clockwise until snug. Do not over-tighten.

Place the microcarrier launch assembly in the top slot inside the bombardment chamber (Figures 4.11 and 5.1).

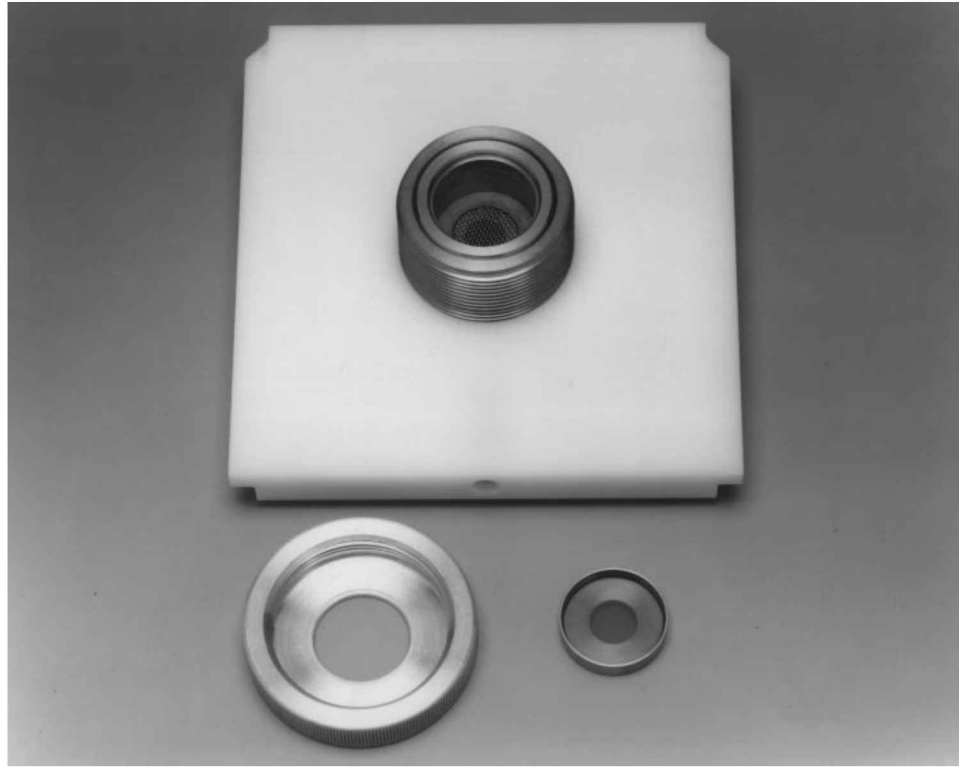


Fig. 4.9. Placement of stopping screen inside fixed nest with macrocarrier and cover lid removed.



Fig. 4.10. Removal / replacement of macrocarrier cover lid with assembled fixed nest. Macrocarrier holder (with macrocarrier properly inserted) is inverted and placed atop fixed nest.

9. **Target cells/tissue placement in chamber**

Place the Target Shelf at the desired level inside the bombardment chamber. Place the sample (usually contained within a Petri dish) on the Target Shelf. Close and latch the sample chamber door.

10. **Chamber evacuation/hold**

Turn on the vacuum source. Set the vacuum switch on the PDS-1000/He (middle red control switch, Figure 4.11) to the **VAC** position. Evacuate the sample chamber to the desired level, at least 5 inches of mercury. The rightmost red control switch (the **FIRE** switch) will be illuminated when the minimum vacuum is achieved.

When the desired vacuum level is reached, hold the chamber vacuum at that level by quickly pressing the vacuum control switch through the middle **VENT** position to the bottom **HOLD** position.

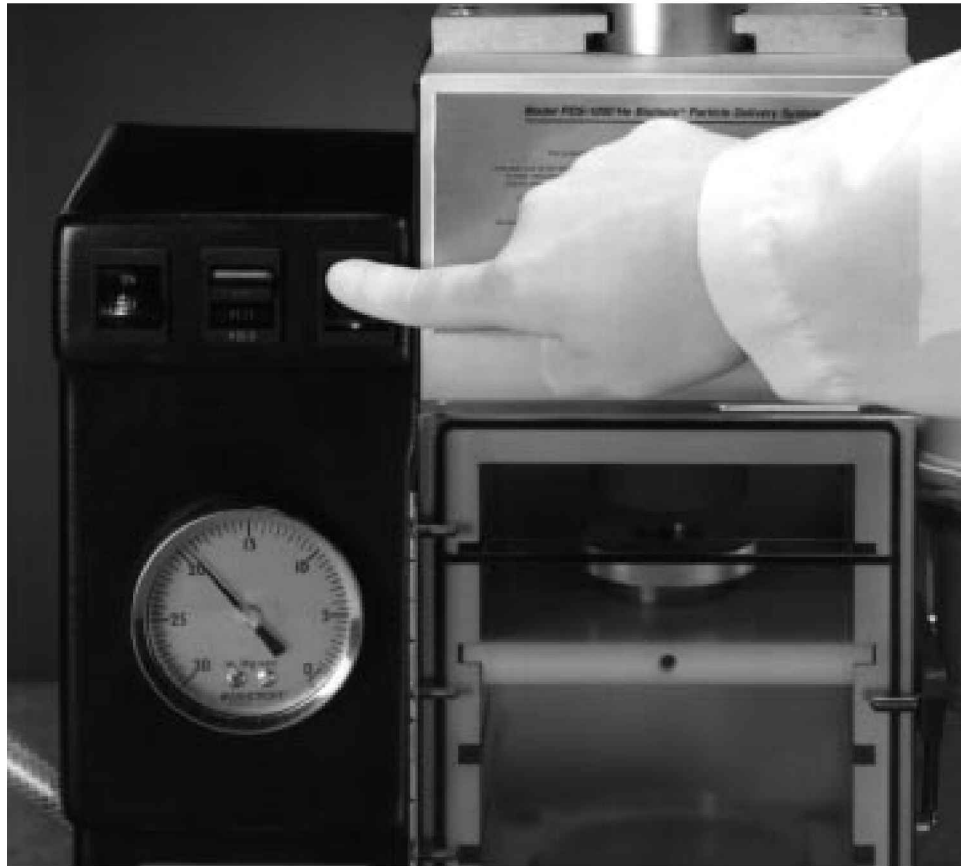


Fig. 4.11. Sample bombardment: power switch ON, vacuum switch on HOLD position, and user continuously pressing FIRE button.

11. **Bombard the sample**

With the vacuum level in the bombardment chamber stabilized, press and hold the **FIRE** switch to allow helium pressure to build inside the gas acceleration tube that is sealed by a selected rupture disk (Figure 4.11).

Estimate rupture disk burst pressure by observing the helium pressure gauge at the top of the acceleration tube. A small pop will be heard when the rupture disk bursts. The rupture disk should burst within 10% of the indicated rupture pressure and within 11–13 seconds.

Release the **FIRE** switch immediately after the disk ruptures to avoid wasting helium. Releasing the **FIRE** switch prior to disk rupture will vent the gas acceleration tube via the 3-way helium metering (solenoid) valve.

Note: Variation in the burst pressure indicated on the helium pressure gauge (on the top of the unit) from the rated rupture disk pressure may be observed if the gas acceleration tube fill rate is improperly set. See Section 5.4 for solenoid valve adjustment procedure.

12. Release vacuum from chamber

Release the vacuum in the sample chamber by setting the **VACUUM** switch to the middle **VENT** position.

13. Target cells removal from chamber

After vacuum is released, the vacuum gauge should read 0 inches of mercury (Hg) of vacuum. Open the sample chamber door. Remove the sample and treat as appropriate.

14. Macrocarrier and stopping screen removal from microcarrier launch assembly

Remove the microcarrier launch assembly. Unscrew the lid and remove the macrocarrier holder. Discard the used macrocarrier and stopping screen (Figure 4.12).

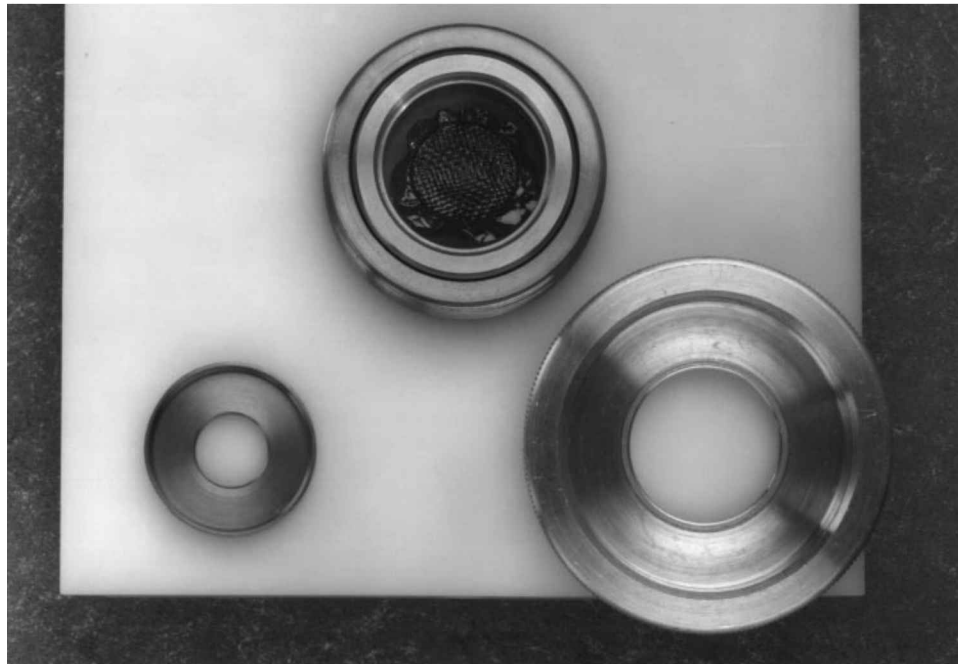


Fig. 4.12. View of used macrocarrier and stopping screen within disassembled microcarrier launch assembly after a bombardment.

15. Removal of spent rupture disk

Unscrew the rupture disk retaining cap from the gas acceleration tube. Remove the remains of the rupture disk (Figure 4.13). The next bombardment may now be performed (from step 7).

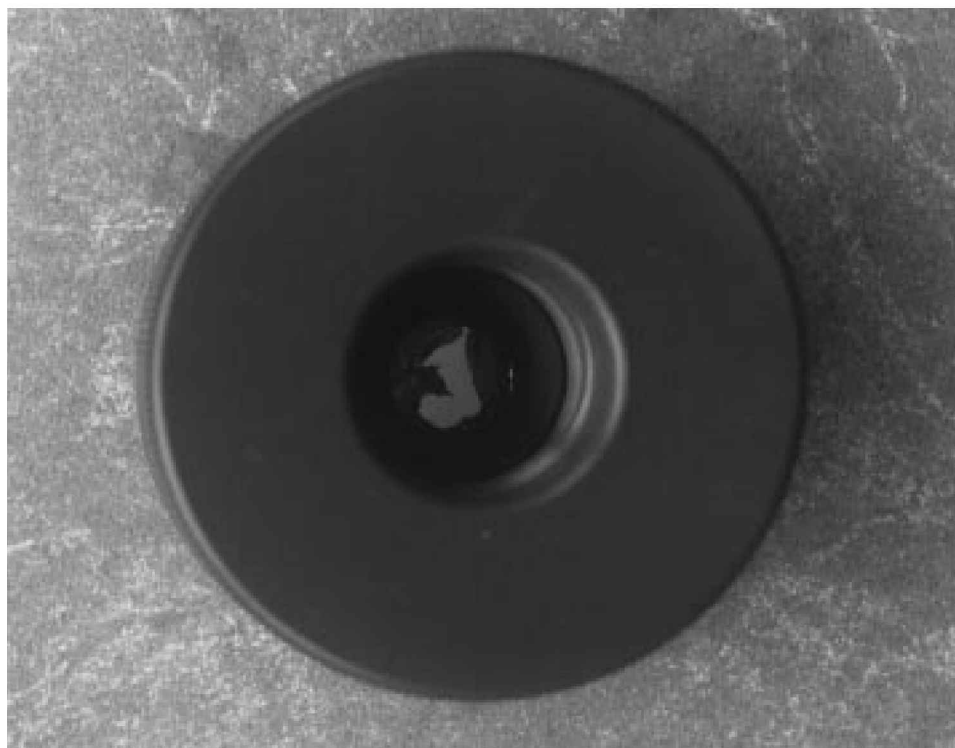


Fig. 4.13. View of spent rupture disk within the retaining cap.

4.3 Removal of Residual Helium Pressure—Shut Down

After completing all bombardment(s), remove the helium pressure from the PDS-1000/He system and close the helium cylinder valve. Perform the following steps to remove helium pressure from the system.

1. Close the helium cylinder valve and chamber door.
2. With at least 5 inches of Hg of vacuum in bombardment chamber, remove residual line pressure from the regulator, solenoid and PEEK tubing by activating the **FIRE** button.
3. Release the **FIRE** button on the apparatus, and remove all tension on the pressure adjustment screw of the helium regulator, turning counter-clockwise, until it turns freely.
4. Vent any residual vacuum from the bombardment chamber by setting the vacuum switch to the **VENT** position.

Section 5 Selection and Adjustment of System Bombardment Parameters

5.1 Overview—Matrix of Variables

Factors affecting bombardment efficiency are numerous, and interact in complex ways. All possible variables cannot be addressed in a single large factorial experiment. Instead, most users will find it sufficient to prioritize variables by the magnitude of their effects, optimizing them individually, and then testing their interactions on a more limited scale. Table 5.1 shows some examples of conditions used in published protocols for a variety of target materials.