Operating Instructions

General

Monitoring cell lysis. The BeadBeater will disrupt over 90% of the cells in about 2-5 minutes of operation. The homogenization procedure involves cell "cracking" action rather than high shear. After homogenization, cell membranes may still appear to be intact when viewed under a microscope. Therefore, to monitor the time course of cell breakage, rely on assay methods that measure intracellular constituents (e.g., OD260, enzyme activity, protein staining, PAGE). If the goal is to isolate intact intracelluar organelles, use the same size beads as suggested for cell disruption (see Beads). Beadbeating is a very effective for this purpose. To maximize the "intact-ness" of the organelles, homogenize for a shorter period of time to get about 70% maximum cell disruption. A homogenization time study of 1, 2, 3, and 5 minutes would be instructive.

Temperature control. The homogenate will be warm after three minutes of "BeadBeating". When isolating proteins, membranes or organelles, cooling may be necessary. When isolating nucleic acids in aggressive extraction media containing phenol/chloroform, guanidinium salts, and/or detergents, temperature control is usually not necessary. The easiest way to minimize heating is by operating the BB, with the ice water jacket installed, for one minute and then let the homogenate sit for one minute, cycling thus until homogenization is complete. Also, consider replacing the clear polycarbonate chamber with a stainless steel closed chamber (accessory, #60801) for much better heat transfer to the ice water. For an even more stringent cooling technique see Methods in Enzymology, Vol.182, p.162-164.

Bead size selection. The correct size beads are 0.1 mm dia. for bacteria, 0.5 mm dia. for yeast, and 1.0 mm dia. or 2.5 mm dia. for chopped-up plant or animal tissue. While glass bead media is most commonly used, denser bead media is available for tough materials. A list of bead media is located at our web site: http://www.biospec.com. Click on Products, then on Beads.

Typical Operating Conditions

Fill the chamber at least 1/2 full (1/2-3/4 is okay) with ice-cold beads and the rest of the volume with cold buffer and biomaterial. Using the standard large chamber, that would be about 200 ml of beads and 200 ml of buffer containing up to 80 g wet weight of cells. Homogenizing cells at lower concentrations is okay but, in the interest of efficient down-stream purification, it may be better to keep cell concentrations high by using a Small Chamber (accessory, #110803) designed to process 15 or 50 ml of biomaterial. In all cases the chamber must be filled completely to the top, excluding as much air as possible when the rotor assembly, along with its large gray rubber gasket, is lowered into the Large Chamber. Screw on the ice water jacket (holding it up-side-down) to secure the chamber assembly.

Fill the ice water jacket with crushed ice and water and place the chamber on the BeadBeater motor. The bead-chain attached to the side of the motor is only used to hold on Small Chambers.

Operate the BeadBeater for a total of three minutes. At the end of the 3-minute run, the temperature of the homogenate will be about 25 deg C. For cooling considerations, see comments above.

Most of the homogenate can be recovered by simply decanting after the beads settle to the bottom of the chamber. To recover the entire product, one can either pour the homogenate, beads and all, into a Buchner funnel containing filter paper, and suction the homogenate out of the beads or one can attach a glass tube with a sintered glass tip (commonly used to aerate cultures) to a side arm flask and suction out the homogenate directly from the chamber.

Cleaning

Beads can be reused about ten times. Soak them in a solution of lab detergent (a sample is included with your BB, but other detergents are good also). Rinse the beads thoroughly and dry them overnight at 50 deg C in a glass or stainless steel tray. Beads can be autoclaved or baked, if desired.

Do not let a "dirty" chamber sit around. Residual cell homogenate is remarkably corrosive and will lead to jamming and leaking of the chamber. Hand wash the plastic rotor assembly and chamber promptly. See below...

Other Things Not To Do

· Do not fill the polycarbonate chamber more than ¾ full with beads. Sample heating will be excessive and the motor may burn out. On the other hand, the chamber must be at least ½ full of glass beads to get good cell disruption.

· Do Not use beads larger than 2.5 mm diameter with the Large Chamber nor larger than 1mm diameter with the 15 ml Small Chamber.

· Do Not use other vessels (Mason jars, etc.) with the BeadBeater. These vessels will not achieve good homogenization and, if made of glass, may break and cause injury. BioSpec Products has extensive experience with the use of continuous bead-mills capable of processing much larger quantities of cells. We would be happy to discuss your project with you.

· Do Not use organic solvents in the chamber. The plastics in the chamber may be attacked. Furthermore, sparks from the BeadBeater motor might ignite leaking solvent.

Cleaning BeadBeater Chambers

A "trick" for thorough cleaning of BeadBeater chambers: The rotor/shaft, a part of the BB homogenization chamber, is temporarily removed from its black plastic bushing unit. In doing so, you can remove any glass fragments by washing the shaft and bushing area.

1) Holding the white Teflon rotor with your fingers so that it cannot rotate, invert the chamber and unscrew the black rubber clutch (the six toothed engaging wheel which mates to a similar clutch on the motor shaft). It has a left-handed screw thread, so push on the slanted side of the teeth on the clutch (i.e. turn clockwise). Hopefully the clutch will come off without much effort. We apply grease to the clutch threads on assembly, but if one does not initiate this special cleaning procedure on a "new" unit, it soon becomes impossible to unscrew the clutch as described.

2) Remove the gray fiber washer. When reassembling the chamber, this washer must be positioned completely over the shaft of the rotor/shaft assembly before screwing on the clutch.

3) Now you can lift out the rotor/shaft assembly out of the bushing unit. Clean everything using a detergent solution, rinse with water, blot dry and reassemble. It is only necessary to "finger-tighten" the black rubber clutch. About every tenth time, lubricate the bronze bushing of the black plastic bushing unit with a single drop of mineral oil. Mineral oil is inert and will not contaminate your homogenate.