BeadBeater

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 yield of intact 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. Additional information on bead media is located at Beads.

Typical Operating Conditions

1) Fill the chamber at least 1/2 full with ice-cold beads and the rest of the volume with cells suspended in cold extraction media. Using the standard large chamber, that would be about 200 ml of beads and 200 ml of buffer containing 1-80 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 cells. In all cases the chamber must be filled completely to the top, excluding as much air as possible as you lower the rotor assembly (don't forget the large gray rubber gasket!) into the Large Chamber. Screw on the ice water jacket (holding it up-side-down) to seal the chamber assembly. If temperature control is not a concern use the metal ring (it looks like a Mason jar lid) to seal the filled chamber instead of the ice water jacket.

2) 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 down

the Large Chamber when it is used without the ice-water jacket.

3) Operate the BeadBeater for a total of two-three minutes. For cooling considerations, see Temperature Control comments above.

4) The homogenate can be recovered by simply decanting. 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 suck out the homogenate directly from the chamber.

Cleaning

Beads can be reused about ten times. Soak them in a solution of lab detergent commonly used for glassware. 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. If you want to be sure your beads are free of all nucleic acids or nucleases, soak the beads for 5 minutes in a 1:10 dilution of ordinary household bleach solution.

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. Also see cleaning details below...

Things Not To Do

- Do Not fill the polycarbonate chamber more than ¾ full with glass or ceramic 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 beads in order 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 or 50 ml Small Chamber. Steel beads cannot be used - they are too heavy to be agitated.
- Do Not use larger vessels (Mason jars, etc.) with the BeadBeater. These containers 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 multi-liter quantities of cell suspension. We would be happy to share our experience with you.
- Do Not use flammable solvents in the chamber. The polycarbonate plastics in the chamber may be attacked. Furthermore, sparks from the BeadBeater motor might ignite leaking solvent or fumes.

Cleaning BeadBeater Chambers

Empty the beads from the chamber into a labware detergent solution. They can be reused many times. Prompt washing of the chamber will remove corrosive biomaterials that can attack the metal parts of the BB chamber.

Here is a method for thorough cleaning of BeadBeater chambers. It takes a minute or so more than simply washing out the intact chamber but will assure that the chamber components last

longer. The rotor/shaft part of the BB homogenization chamber is temporarily removed from its black plastic bushing unit. In doing so, you will be able to remove any glass fragments that might have worked their way into 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). The shaft 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 silicone grease to the clutch threads on assembly, but if the enhanced cleaning procedure is not done soon after receiving a "new" unit, it soon becomes impossible to unscrew the clutch.
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, put a light coat of silicone grease on the threads of the shaft which engage the clutch and lubricate the bronze bushing in the center of the black plastic bushing unit with a single drop of mineral oil. Mineral oil is inert and will not contaminate your biopreparation.

Beads

For Cell Disruption we suggest:

Size

- When working with Bacteria use the 0.1mm diameter glass beads.
- When working with Yeast/Fungi use the 0.5mm diameter glass beads.
- When working with Most Tissue use the 1.0mm diameter glass beads or zirconia/silica beads.
- When working with Skin or "soft" plant material use a 2.0mm diameter zirconia beads.
- When working with really tough or fibrous tissue use the same sized beads (see above) but choose a more dense bead material. For example, researchers prefer 0.1mm zirconia-silica beads for disruption of spores or 2.3 mm chrome-steel beads for extraction of tough fibrous plant material like monocot leaves. Some users have had good results using the MiniBeadbeater in a "dry grinding" process at liquid nitrogen temperatures.

Density

- Glass has a density of 2.5 g/cc (most commonly used bead media for "Beadbeating")
- Zirconia/Silica has a density of 3.7g/cc (50% more dense than glass - good for spores and most tissues)

- Silicon Carbide (sharp particle, not a bead) has a density of 3.2 g/cc (May work faster on tissue samples because the particles have sharp cutting edges. Their utility is still under investigation, but see Brein's comments below)
- Zirconia has a density of 5.5g/cc (100% more dense than glass - good for tough tissue)
- Steel, stainless or not, has a density of 7.9g/cc (used mostly for grinding leaves - stainless steel beads are expensive and but can be reused. Chrome steel beads are 10X cheaper - cheap enough to be use one time and thrown away.)
- Tungsten Carbide has a density of 14.9g/cc (While very dense, this bead is not recommended for biopreps - leaves prep dirty)

Other uses for beads:

- Quick and easy plating of yeast and bacteria. Add a few sterile 6.3mm diameter glass beads to the plate to evenly spread yeast and bacteria inoculums. No need to remove them afterward.
- Pack roller bottles, tubes or vials with 6.3 mm diameter glass beads to greatly increase the surface area for tissue culture growth. In this application the beads should be packed tightly to prevent movement of the beads during rolling or shaking. When growing cell in non-agitated culture flasks, confluence is reached faster and cell density is enhanced by adding a layer of 0.1 mm diameter beads (see Growth of Three Established Cell Lines on Glass Microcarriers by James Carani, et. al., Biotechnology and Bioengineering, Vol.25, p.1359-1372 (1983).
- Create a bio-reactor by packing a column with glass beads and inoculate with select surface-adhering micro-organisms or cells.
- Keep dialysis tubes vertical during dialysis by adding a few large beads before sealing.