What is Bead Beating?

Bead beating is a homogenization process used to break up (lyse) samples in order to release the DNA, RNA and proteins contained within the cells. Samples are placed in tubes with the appropriate grinding beads and subjected to high energy mixing. The beads impact the sample, eventually breaking it down on the cellular level releasing subcellular contents. The samples are then typically centrifuged and the lysate recovered from above the beads.

Instruments

The Benchmark *BeadBug™* and *BeadBlaster™ 24* are bead beating homogenizers, designed to rapidly homogenize small samples in disposable tubes with the aid of grinding beads. Both instruments work by physically moving tubes, samples, and grinding beads in an oscillating motion several thousand times per minute. Through this motion, the beads tear into and crush samples on impact. They are different in capacity - if only a few samples require processing a day, then the BeadBug will be sufficient for most researchers. For those with higher capacity demands or a larger lab group, the BeadBlaster 24 is more practical.

BeadBeating Tips

Bead beating can be effective at disrupting many different sample types. The key aspect of successfully homogenizing samples is to match the correct bead to the sample and then not to overload the disruption tube. For bead beating to be effective, the beads need to move and crash into the sample. Tubes packed with solid tissues and/or filled with buffer prevent the beads from moving freely.

General guidelines that can help to ensure that samples are homogenized effectively include:

- Do not over fill tubes. The total volume of the sample, beads, and buffer should never exceed half the volume of the tube. Less is better.
- Sample and buffer should be twice the volume of the beads.
- Detergents should be kept to a minimum as the foaming will impede the movement of the beads.
- Solid tissues should never be more than 1/20th the volume of the disruption tube, i.e., no more than 100 mg tissue in a 2 ml tube.
- If sample warming is an issue, process the sample in short bursts.

Prefilled tubes

A variety of prefilled tubes are available for homogenizing samples including those with glass and Triple-Pure™ zirconium beads. Both bead types are acid washed and processed to remove nucleases and proteases. The Triple-Pure bead tubes undergo special handling during filling and are quality tested to ensure the elimination of DNase, RNase, protease, and nucleic acids. When samples are used for molecular biology applications, the high purity of the Triple-Pure beads is preferred.

Both glass (silica) and zirconium beads are effective at disrupting cells and tissues. The added density of zirconium helps to yield better homogenized samples. Benchmark has nine varieties of prefilled tubes, each which can be used with a general category of sample. These tubes are:

Standard Glass Beads, 0.1 mm – Effective for the homogenization of bacteria. Best used for cultured microbes where low cell densities are not an issue.

*Standard Glass Beads, 0.5 m*m – For larger microbial cells, like yeast. Effective for homogenizing yeast cultures, especially where heat labile proteins might be involved.

Standard Glass Beads 1.0 mm – For small pieces of soft tissue, fungal mycelium, and larger algal cells.

Triple-Pure Zirconium Beads, 0.1 mm – For all bacteria and low density cultures.

Triple-Pure Zirconium Beads, 0.5 mm –Effective for the homogenization of yeast and smaller algae cells. Effective for low density cultures and samples where contamination is an issue.

Triple-Pure Zirconium Beads, 1.0 mm – Use for fungal mycelium, soft tissues, very small tissue samples.

Triple-Pure Zirconium Beads, 1.5 mm – Effective for homogenizing softer animal tissues (adipose, mouse intestine, hypothalamus, and liver) as well as leaf and soft vegetables (e.g., potato).

Triple-Pure Zirconium Beads, 3.0 mm – Provides greater energy for shearing and rupturing tough samples such as muscle, lung, and kidney as well as small plant seeds, stems, and roots.

Stainless Steel Beads, 2.8 mm – Stainless steel has greater density than both glass and zirconium and can be used on very tough samples such as cornea and connective tissue and in situations where the other tube types are not effective. Metals beads are effective at homogenizing tough samples, but they also generate significant heat. Care should be taken when working with heat labile molecules. Plant extracts will often oxide rapidly in the presence of stainless steel.

Sample Types

Bacteria – As bacteria tend to be very small, 1-3 µm in length or diameter, smaller beads normally work better for cell disruption. A culture of bacteria contains upwards of 109 cells/ml thus smaller beads (e.g., 0.1 mm) allow for greater surface area for impacting cells. This is a very significant factor in cracking bacteria. Generally the mass of the beads greatly exceeds the mass of the bacteria, thus both glass and zirconium work well. However, glass beads tend to clump more than denser zirconium beads which settle more effectively in lysates after processing. Other organisms of comparable size, such as cyanobacteria, can also be processed in a similar manner.

Yeast – The next larger microorganism after bacteria is yeast, being roughly 10+ microns in diameter or length. Beads with a diameter of 0.5 mm tend to work very well on yeast cells. Many single cell algae can be processed as if they are yeast.

Filamentous Fungi – Fungi that are not solid bodies, such as pycnidia and similar fruiting bodies, are effectively disrupted with the next size of beads. Beads of 1.0 mm are very effective for shearing mycelium.

Plant Leaves – Leaves can be homogenized readily using 1.5 and 3.0 mm zirconium beads and 2.8 mm stainless steel beads. Leaves are often harvested by cutting out the sample with a paper hole punch. A sample of this size is normally 10-20 mg in mass, thus multiple punches can be processed in one tube.

Shoots and Stalks – More resilient plant material can be effectively homogenized using the larger bead sizes. Stalks of bundled grasses and young roots and shoots can be effectively homogenized using these beads, however plants which are more mature may have significant pulp following homogenization if their lignocelluloses content is high. Sample size should not exceed 50-70 mg to ensure good disruption.

Seeds – Small fresh seeds can be processed effectively using 3.0 mm zirconium and 2.8 mm stainless steel beads. Larger seeds must first be dissected so that the sample mass is no more the 50 mg.

Soft Animal Tissue – Animal tissues can be grouped into soft and hard tissue types. The softer tissues, such as liver, adipose, and hypothalamus, can readily be disrupted using 1.5 mm beads or larger. Though easily homogenized, it is still important not to overload tubes with soft animal tissues.

Resilient Animal Tissues – Fibrous animal tissues such as muscle, lung, kidney and heart can be difficult to homogenize. By using larger beads, these tissues can be effectively disrupted to liberate RNA, DNA and proteins with very high yields. Triple-Pure 3.0 mm zirconium beads and stainless steel beads (2.8 mm) are effective for disrupting these tough tissues. Sample size is best kept under 50 mg while buffer should be no more than 200-300 µl. Many RNA extraction protocols use larger volumes of buffer, and for these processes the sample can be homogenized in less extraction buffer and then brought up to volume following homogenization.

Prefilled Disruption Tubes

D1031-01	Standard glass, 0.1mm dia, pk 50
D1031-05	Standard glass, 0.5mm dia, pk 50
D1031-10	Standard glass, 1.0mm dia, pk 50
D1032-01	Triple-Pure Zirconium, 0.1mm dia, pk 50
D1032-05	Triple-Pure Zirconium, 0.5mm dia, pk 50
D1032-10	Triple-Pure Zirconium, 1.0mm dia, pk 50
D1032-15	Triple-Pure Zirconium, 1.5mm dia, pk 50
D1032-30	Triple-Pure Zirconium, 3.0mm dia, pk 50
D1033-28	Stainless steel, 2.8mm dia, pk 50
D1032-SK	Starter kit, 10 ea of 0.1, 0.5, 1.0, 1.5, 3.0mm dia

BeadBlaster™ 24
D2400

BeadBug™
D1030

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