**Procedure summary**

This procedure describes the use of the Biospec Mini-Beadbeater-24 found at LCTS. Beadbeating is used for complete cell lysis e.g. during nucleic acid or protein extraction. At LCTS it is also used to break up flocks of algae etc. in pond samples prior to using the FlowCAM.

**Related Procedures**

| | |
|---|---|
| FlowCAM | LC-06-001-012 |

**Procedure impacts and concerns**

| | |
|---|---|
| Safety | Proper PPE for this procedure: safety glasses, safety toe shoes and gloves. Nitrile gloves should be worn when handling pond samples. The MSDS/SDS for chemicals used in this SOP should be reviewed. |
| Quality | NA |
| Delivery | NA |
| Environmental | Local policies and procedures should be followed as determined by the site leadership. |
| Cost | NA |
| Compliance | Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required (see 29 CFR 1910.120 and 1200). |

**Responsibilities and owners**

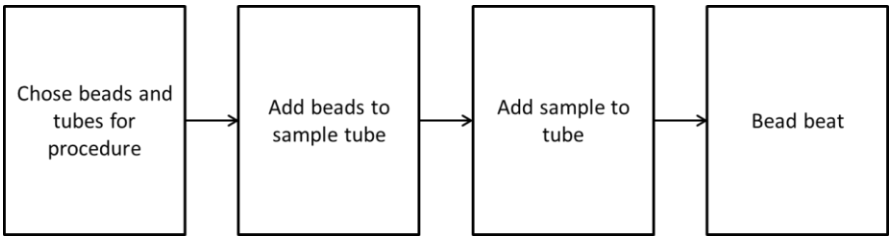
| | | |
|---|---|---|
| Document Owner | Manage content and distribution | |
| Process Owner | Responsible for content and process validation | |
| Plant Manager | Responsible for implementation and conformance | Becky Ryan |

**Process**

**2.1 Process description**

The Mini-BeadBeater-24 disrupts microbial cells and plant and animal tissue by violently agitating four to twenty-four 2 ml screw-cap microvials containing small glass, ceramic, or steel beads and 0.1 to 1 ml disruption buffer. Even resistant samples like yeast, spores or fibrous tissue are completely homogenized in 1-3 minutes. The non-foaming, aerosol-free method preserves enzymes and organelles. In the presence of nucleic acid extraction media such as phenol, Gu-SCN or a commercial kit solution, DNA or RNA is recovered in the highest possible yield. The method is ideal for PAGE, PCR applications, and diagnostics using antibody or oligonucleotide probes.

**2.2 Process diagram: Work Instruction**




**2.3 Process steps**

**1. Choose beads for application.**

The table below summarizes the manufacturer's recommendation for bead size selection.

| Sample type | Typical bead size |
|---|---|
| Bacteria | 0.1 mm |
| Yeast, fungi, tissue culture cells | 0.7 mm |
| Plant / animal tissue | 2.5 mm |

Up to 400 mg (wet wt) of biomaterial can be disrupted per ml of extraction media. In most applications, beads made of glass or zirconia-silica give excellent results. In special cases (grinding dry leaf material, wet grinding soaked seeds, disrupting skin or cartilage) beads made of denser material such as zirconia or steel may be required.

**2. Chose tubes for application.**

a. Use screw-cap microtubes with integral o-ring seals in order to eliminate aerosol formation during the homogenization.
b. Label tubes with sample names prior to use

**3. Add beads to sample tube**

a. For lysing cells, fill the screw-cap vial at least 1/2 full (1/2-3/4 is okay) with beads.

For other applications of beadbeating: different bead size/material, ratios of beads to sample, beating times, buffer composition etc. may be used as determined experimentally (see table for guidelines).

| Application | Amount of beads | Sample volume | Beating time |
|---|---|---|---|
| FlowCAM sample prep to disrupt flocks | ~0.5g 0.7 mm zirconium beads | 1ml | 3 sec |
| Cell lysis | Fill tube 1/2-3/4 full | Fill tube | 2-3 min |
| Cell lysis that requires chilled samples | Fill tube 1/2-3/4 full | Fill tube | 1 min beating, 1 min in ice bath. Repeat for total beat time of 2-3 min. |

**4. Add sample to tube**

a. Add extraction media and cells, being sure to fill the microtube almost to the top. Exclude as much air from the microtube as possible. Any air in the tube may decrease lysis efficiency and/or sample integrity.
b. Be sure there are no beads on the threads of the microtubes when screwing down the cap

Figure 1 Beadbeater and control dials

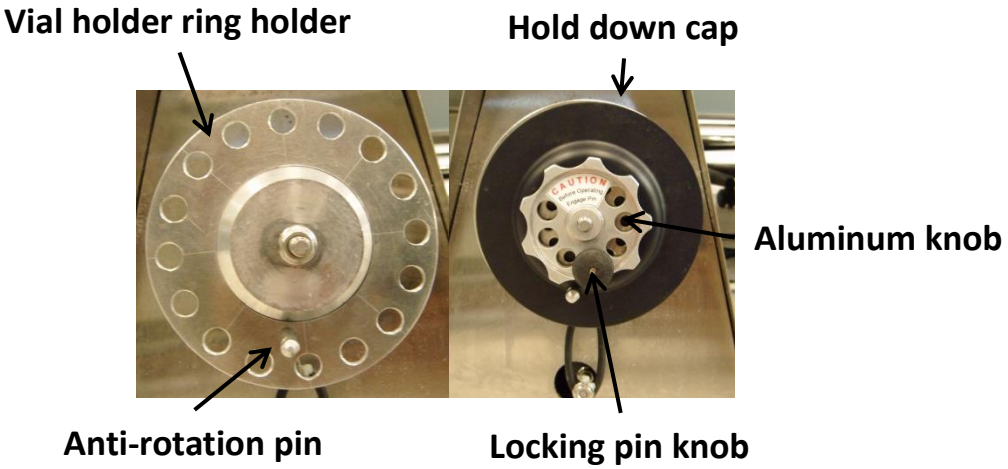


Figure 2 Vial holder and wiggle mechanism assembly

**5. Bead Beat**

a. Raise safety cover
b. Raise locking pin by pulling and twisting slightly
c. Unscrew and remove aluminum knob
d. Remove hold down cap
e. Ensure anti rotation pin is aligned with the hole in vial holder ring holder as shown in figure 2
f. Load 1 to 24 microtubes into the vial holder ring holder. Distribute them symmetrically as you would do with a centrifuge. If using less than 4 sample vials, insert vials so that at least four vials are in the holder. All tubes should be balanced (i.e. tubes of equal volume/weight opposite each other). Unless using all holes, leave two balanced tubes in the holder to ensure that there are always 4 tubes present.

g. Place the loaded vial holder ring on the aluminum wiggle head (the latter being attached to the motor). Rotate the loaded vial holder ring to a position where the hole in the vial holder ring is aligned with the anti-rotation pin sticking out of the wiggle head. Slide the vial holder ring down the pin and seat it on the wiggle head. Next, align the large, black plastic hold-down cap and slide it down to contact the tops of the microtubes.
h. Screw on and firmly hand tighten the aluminum knob. To do this, the locking pin, which is part of the knob, must be in the down position. A slight twist of the pin keeps it in either a down or raised position. As you tighten the aluminum knob a clicking sound will be heard as the locking pin engages holes in the black hold-down cap.
   IMPORTANT! The locking pin is an important safety feature. Test that the pin has engaged into one of the ring of holes on the hold down cap. To do this, attempt to further tighten the knob. Do not proceed if the cap does not lock. Raise the pin on the aluminum knob and repeat the tightening, locking and testing process. Failure to sufficiently tighten the aluminum knob or to engage the locking pin can result in rapid destruction of the central shaft of the aluminum wiggle head.
i. Lower the safety cover
j. Set the timer. A typical setting for cell disruption is 2-3 minutes. (Note: If you are working with heat-sensitive material, consider homogenizing for a shorter period, e.g. 1 minute, then remove the vial holder with its vials and cooling the vials in ice-water for 1 minute. Cycle thus, for a total runtime of three minutes. No cooling is needed for nucleic acid extraction providing an appropriate nucleic acid extraction media/buffer is being used).
k. Start the machine by pressing the start/stop button. The timer resets itself automatically at the end of the run.
l. To remove the vials and vial holding ring, first raise the locking pin to the up position (a slight turn of the locking-pin knob will keep it in the raised positon). Unscrew the aluminum knob, remove the black vial hold down cap and, finally the vials in their vial holding ring.

**Document control**

Revision history

| | |
|---|---|
| R0 – Philip Lee | 11/19/2014 |

Document approval
Becky Ryan

Document reviewers

| | |
|---|---|
| Phil Lee | 11/19/2015 |
| Chris Meenach | 11/20/2014 |
| Mike Burnett | 11/19/2014 |
| Raechel Roberts (safety committee) | |