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1.1 Bead beating

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Sample preparation for Western bolt

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://www.biospec.com/instructions/minibeadbeater_8

PROTOCOL CITATION

Elizabeth Fozo 2020. 1.1 Bead beating. **protocols.io** https://protocols.io/view/1-1-bead-beating-bqqmmvu6

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ABSTRACT

Sample preparation for Western bolt

BEFORE STARTING

BEFORE STARTING

6/27/17 (revised 2/2/18)
#bikash
#Protein extraction

Sample preparation for Western blot

1 Bead beating

1.1 Bead size selection

- 0.1 mm diameter for bacteria, 0.5 mm diameter for yeast, and 1 or 2.5 mm diameter for a chopped-up plant or animal tissue.
- Fill2 ml screw-cap microtube with0.5 gm of beads, keep the tubes in ice and add cells. Use screw-cap microtubes with integral o-ring seals in order to eliminate aerosol formation during the homogenization. Be sure there are no beads on the threads of the microtubes when screwing down the cap.

1.2 Operating the Mini-BeadBeater-8

- Insert one to eight microtubes into the chamber holder. Distribute symmetrically. Screw in the black plastic chamber cap, flat side down, until it is in contact with the tops of the microtube caps. Finally, screw on tight the white nylon wing nut.
- Set the toggle switch on the control box to 'Time' and select the speed. A typical setting for cell disruption is 3 minutes at Homogenize. If you are working with a heat-sensitive material, consider homogenizing for 1 minute, then cooling the vials in ice-water for 1 minute. Cycle for a total 'On' time of three minutes. Nucleic acid extraction does not need cooling.
- Start the homogenization by pushing the white button in the middle of the timer dial. The timer dial resets itself automatically at the end of the run.

CAUTION - While the MBB-8 is running, changing the Timer settings will damage the timer. If you must change the time setting, press, and hold down the white start button while changing the position of the timer dial.

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1.3 Safety Concerns

- Operate the MBB-8 with the black plastic hood over the chamber. This prevents the user from coming in contact with the shaker during operation and will help trap anything that might break free.
- The **noise level of the disrupter exceeds 85dB**. You may want to isolate the MBB-8 to a side room. Do not isolate the MBB-8 in a cold room. It will lead to premature motor failure and does not help to keep samples cold during homogenization.
- Centrifuge at 10,000 g for 5 minutes. Transfer the supernatant to an Eppendorf tube and spin again (10,000g for 5 minutes). Transfer the supernatant to a polypropylene microfuge tube(which is your total protein).