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Protocol for Using the Microfluidizer Lysis Apparatus

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ABSTRACT

The Microfluidizer Lysis apparatus is used to efficiently lyse bacterial and yeast cells using high pressure sheering against a ceramic cone inside the machine. Unlike conventional approaches for cell lysis such as sonication or French pressing, the microfluidizer is able to efficiently lyse your cells quickly and efficiently, only taking approximately 1 – 2 minutes. Cells can be passed through the Microfluidizer multiple times and the pressure of the machine can be adjusted based on cell type. Note, however, that the Microfluidizer does not work efficiently on mammalian cells.

PROTOCOL CITATION

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1 Begin by filling the red tray next to the Microfluidizer with a ice. Pack the ice densely around the sheering vessel/compartment, as shown in Figure 1. The sheering vessel flows your cells at ultrasonic speeds against a pointed ceramic cone. This quickly and efficiently sheers your cells, but it also heats your sample up a bit. To minimize
heat exposure, you should make sure that as much of the vessel is packed tightly with ice prior to use. Further, after packing the shearing vessel with ice, let it cool for 20 – 30 minutes prior to use.

Figure 1: Shearing vessel and ice box

2 When you first use the Microfluidizer, you will see some liquid in the sample chamber, as shown in Figure 2. This is the storage solution – 70% isopropanol. Before you add your sample to the sample chamber, you will want to wash out the sample chamber extensively with water. To do so, flip the valve as shown in Figure 3. This will start the machine. Note that when you are washing the sample chamber, never let air get into the machine. You should never let the machine run if the chamber is empty/dry. Always make sure that liquid is flowing through the machine to prevent air bubbles from getting into the system.

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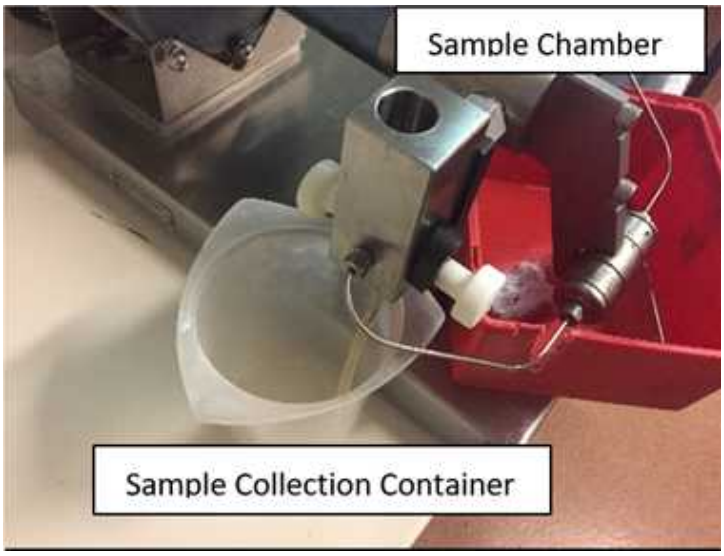

Figure 2: Sample chamber and exit tube

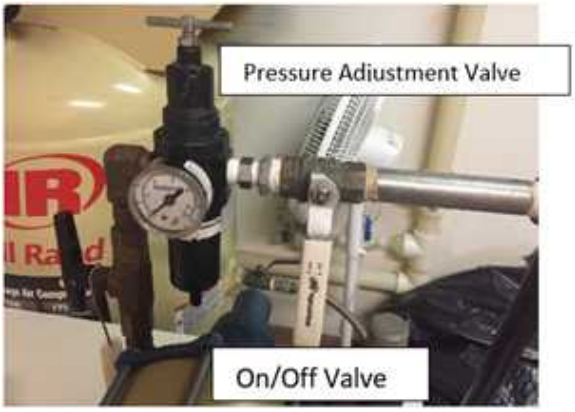

Figure 3: On / Off valve and pressure valve

3 After you have washed the sample chamber with water, you will want to equilibrate the valves with your sample buffer. You will want to collect the waste from your washes in a container below the sample chamber, as shown in Figure 2. When you are collecting your lysed sample, it is a good idea to have a container that is also chilled on ice.

4 After washing the sample chamber and equilibriated it with your Sample buffer, switch the waste vessel and place the chilled collection Beaker (on ice) under the collection nozzle.

5 Then begin to flow through your sample by slowly adding it to the sample chamber. Remember to never let the sample chamber become empty. After you have finished adding your sample to the

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sample chamber, add water to the sample chamber until the flow through is clear.

6 After you have collected your sheared sample, you may flow it through the Microfluidizer again by simply adding it back to the Sample Chamber. Once you have sheared your sample sufficiently, make sure to centrifuge it quickly to prevent degradation.

7 Clean the Microfluidizer by washing extensively with water. Then wash with 70% isopropanol and then store in 70% isopropanol and turn off the valve. This means that the Sample Chamber will be filled up with 70% isopropanol.

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