Cheatography

Mechanical Methods of Cell Disruption Cheat Sheet

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Introduction

Cell disruption is a process in which the biological molecules within a cell are released and isolated from the rest of the cell, so they can be analyzed, studied and experimented upon. There are both mechanical and non-mechanical methods of cell disruption. Here are some common mechanical methods

Source: https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/traditional-methods-cell-lysis.html

Bead Method (a.k.a. “Beadbeating”)

With the bead method (a.k.a. “beadbeating”), very small beads (0.1-6 mm in diameter) made of glass, ceramic or steel are mixed with a sample that has been suspended in aqueous media (i.e. a solution in which the solvent is water). This process shears open the cell wall, yet in a manner that is gentle enough to ensure that the biological molecules within the cell remain intact

Sonication

Sonication is the third class of physical disruption commonly used to break open cells. The method uses pulsed, high frequency sound waves to agitate and lyse cells, bacteria, spores and finely diced tissue. The sound waves are delivered using an apparatus with a vibrating probe that is immersed in the liquid cell suspension. Mechanical energy from the probe initiates the formation of microscopic vapor bubbles that form momentarily and implode, causing shock waves to radiate through a sample. To prevent excessive heating, ultrasonic treatment is applied in multiple short bursts to a sample immersed in an ice bath. Sonication is best suited for volumes <100mL..

Grinding

Manual grinding is the most common method used to disrupt plant cells. Tissue is frozen in liquid nitrogen and then crushed using a mortar and pestle. Because of the tensile strength of the cellulose and other polysaccharides comprising the cell wall, this method is the fastest and most efficient way to access plant proteins and DNA.

Blenders

The use of blenders (both high speed or Waring) can be used to disrupt cell walls. This is the same process used by centrifugation, which separates or concentrates materials suspended in a liquid medium.

Freezing

Microwave

Microwave (along with autoclave and other high temperature methods) are used to disrupt the bonds within cell walls, and also to denature proteins. This is a somewhat risky method, as the excess heat can quickly damage the rest of the cell.

Standard Liquid Homogenizers

Liquid-based homogenization is the most widely used cell disruption technique for small volumes and cultured cells. Cells are lysed by forcing the cell or tissue suspension through a narrow space, thereby shearing the cell membranes. Three different types of homogenizers are in common use.:

- A Dounce homogenizer consists of a round glass pestle that is manually driven into a glass tube.
- A Potter-Elvehjem homogenizer consists of a manually or mechanically driven PTFE pestle shaped to fit a rounded or conical vessel. The number of strokes and the speed at which the strokes are administered influences the effectiveness of Dounce and Potter-Elvehjem homogenization methods. Both homogenizers can be obtained in a variety of sizes to accommodate a range of volumes.
- A French press consists of a piston that is used to apply high pressure to a sample volume of 40 to 250 mL, forcing it through a tiny hole in the press. Only two passes are required for efficient lysis due to the high pressures used with this process. The equipment is expensive, but the French press is often the method of choice for breaking bacterial cells mechanically.

Additives & methods for physical disruption

Cells can be treated with various agents to aid the disruption process. Lysis can be promoted by suspending cells in a hypotonic buffer, which cause them to swell and burst more readily under physical shearing. Lysozyme (200 µg/mL) can be used to digest the polysaccharide component of yeast and bacterial cell walls. Alternatively, processing can be expedited by treating cells with glass beads in order to facilitate the crushing of cell walls. This treatment is commonly used with yeast cells. Viscosity of a sample typically increases during lysis due to the release of nucleic acid material. DNase can be added to samples (25–50 µg/mL) along with RNase (50 µg/mL) to reduce this problem. Nuclease treatment is not required for sonicated material since sonication shears chromosomes..

Finally, proteolysis can be a problem whenever cells are manipulated; therefore, protease inhibitors should be added to all samples undergoing lysis.

BEE Laboratory High Pressure Homogenizers

A growing number of life science researchers are choosing BEE Laboratory High Pressure Homogenizers, because they represent a radical departure from conventional equipment and provide more experimentation options and capabilities for cell disruption, as well as emulsions, dispersions and liposomes.

The freeze-thaw method is commonly used to lyse bacterial and mammalian cells. The technique involves freezing a cell suspension in a dry ice/ethanol bath or freezer and then thawing the material at room temperature or 37°C. This method of lysis causes cells to swell and ultimately break as ice crystals form during the freezing process and then contract during thawing. Multiple cycles are necessary for efficient lysis, and the process can be quite lengthy. However, freeze/thaw has been shown to effectively release recombinant proteins located in the cytoplasm of bacteria and is recommended for the lysis of mammalian cells in some protocols..

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Published 29th December, 2018.
Last updated 29th December, 2018.

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