TechTalk I

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Yeast (*S.cerevisiae*)

Disruption Parameters:

The parameters that affect the amount of disruption are the supply gas(N_2 or He) pressure and the viscosity of the medium. Increasing the viscosity by the inclusion of 20% glycerol causes a higher percentage of cell breakage using lower pressures (i.e. 75-100 psi).

Volume:

The minimum volume of cells that can be disrupted at one time is 5 ml. There is no upper volume limit if the BioNebulizer is used in the open drain, single cycle mode or the open drain, continuous mode with auxiliary feed and receiver vessels. However, longer inlet and outlet tubes are required which can be supplied from in-house materials.

Pressure:

The operating pressure range for yeast cell disruption is 150-200 psi.

Protocol:

1. Yeast cells (Fleischmann's baker's yeast) are washed and resuspended in 1 nM phosphate buffer (pH 7.0)to a final concentration of 3.3×10^7 cells/ml.
2. Use 25 ml of the yeast cell suspension for initial testing. Transfer 1.5 ml of the sample to a microfuge tube for untreated control.
3. Run the remaining sample through the BioNebulizer at 200 psi N_2 in the closed drain, single cycle mode for one cycle. Transfer a 1.5 ml sample to a separate tube for later analysis.
4. Repeat for 3 to 5 cycles. Briefly observe the sample microscopically after each cycle to determine the desired end point.
5. Centrifuge the samples at 10,000 x g for 10 minutes at room temperature. Collect the supernatant portions and store at -70°C for later characterization (eg., protein determination).

Results:

Typical Bradford assay results (Pierce- standard protocol) are A_{595} values of 0.26 after one cycle and 0.55 after three cycles.

Operation Notes:

Precooling the BioNeb system is not necessary because the very process of bionebulization causes a cooling of the solution which aids in stabilizing proteins. Temperature reductions of 10° C have been observed. The superior efficiency of disruption means that only half as much sample need be used resulting in significant savings of time and material.

The BioNeb Cell Disruption System cleans easily with soap and water. Ethanol can also be used to clean the unit. **DO NOT USE ACETONE.** The unit is completely autoclavable when sterilization is necessary, however, autoclaving will greatly reduce the life of the unit.

This data represents results observed with production models of the BioNeb System. Some user variability may be expected, depending on specific protocols.

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TechTalk II

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Mammalian Cell Line (*CHO, CHL, Nb2, 3T3, L*)

Disruption Parameters:

The parameters that affect the amount of disruption are the supply gas(N_2 or He) pressure and the viscosity of the medium.

Volume:

The minimum volume of cells that can be disrupted at one time is 5 ml. There is no upper volume limit if the BioNebulizer is used in the open drain, single cycle mode or the open drain, continuous mode with auxiliary feed and receiver vessels. However, longer inlet and outlet tubes are required which can be supplied from in-house materials.

Pressure:

The operating pressure range for mammalian cell disruption is 20-40 psi.

Protocol:

1. Suspend cells from one T-75 culture in 20 ml of PBS (or buffer of choice).
2. Transfer 3 ml of the sample to a separate tube as an untreated control.
3. Run the remaining sample through the BioNebulizer at 30 psi N_2 in the closed-drain, single cycle mode for one cycle. Transfer 3 ml of the sample to a separate tube for later analysis.
4. Repeat for three to five cycles. Briefly observe the sample microscopically after each cycle to determine the desired end point.
5. Centrifuge samples at 1500 x g for 20 minutes at 4°C. Collect supernatant portions and store at -70°C for later characterization (eg., protein determination).

Results:

Cycling of the above referenced mammalian cell lines for 3 minutes in continuous recycling mode normally yields 85% to 100% disruption, as determined by dye exclusion.

Operation Notes:

Precooling the BioNeb System is not necessary because the very process of nebulization causes a cooling of the solution which aids in stabilizing proteins. Temperature reductions of 10°C have been observed. Bionebulization is faster and more reproducible than repeated freeze-thaw. The use of detergents which can destabilize proteins and can be difficult to remove is also avoided.

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TechTalk III

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Yeast (*Saccharomyces cerevisiae*)

Fleischmann's baker's yeast was washed free of starch and resuspended in 1 mM potassium phosphate buffer (pH 7.0) to a final concentration of 3.3×10^7 cells/ml.

Disruption Parameters:

BioNeb System: With reservoir removed, 20 ml of yeast suspension was nebulized under 200 psi N_2 in the closed-drain, single cycle mode for three cycles.

Yeda Press: The cell suspension(20 ml) was passed through the Yeda Press under 1,000 psi N_2 three times. This experiment was repeated using 500 psi N_2 and 200 psi N_2 .

Measurement of Protein:

Samples of 0.60 ml were removed after 0, 1, 2, and 3 cycles of nebulization or Yeda Press treatment. All samples were centrifuged at 10,000 x g for five minutes at room temperature. Protein concentration was estimated by transferring 60 ul of the supernatant portion of each sample to 3 ml aliquots of Bradford (Bradford, M.M. [1976]. Analytical Biochemistry 72: 218-254.) reagent and measuring the absorbtion at 595 nm.

Results:

The A_{595} of the BioNeb System sample was 0.16 after one cycle and reached a peak of 0.33 after two cycles. All of the Yeda Press samples as well as the control samples (no cycles) gave A_{595} values of less than 0.05.

Conclusions:

The BioNeb System was able to quickly and efficiently break yeast cells at 200 psi resulting in the release of significant quantities of intracellular proteins. The Yeda Press was unable to break yeast cells at even 5 times the N_2 pressure.

Operation Notes:

Precooling the BioNeb System is not necessary because the very process of nebulization causes a cooling of the solution which aids in stabilizing proteins. Temperature reductions of 10°C have been observed.

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TechTalk IV

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organisms:

Yeast (*Saccharomyces cerevisiae*)
Bacteria (*Escherichia coli*)

Yeast: Fleischmann's baker's yeast was washed free of starch and resuspended in 1 mM potassium phosphate buffer (pH 7.0) to a final concentration of 3.3×10^7 cells/ml.

Bacteria: *E.coli* (strain OH10B) was incubated at 37°C to stationary phase. The cells were centrifuged 2,000 x g for 20 minutes at 4°C. The pellet was washed twice with Tris-buffered saline (TBS [20mM Tris-Cl, 150 mM NaCl, pH 7.5]) The final pellet was diluted to a cell concentration of 2×10^9 cells/ml.

Disruption Parameters:

BioNeb System: The bottom reservoir was removed and 10 ml of each sample was nebulized under 200 psi He in the open-drain, continuous cycle mode for 3 minutes.

Sonication: Each sample (10ml) was sonicated with the Fisher "Sonic Dismembranator" Model 150 at a power setting of 80% for 3 minutes with no external cooling.

Measurement of Protein:

Samples of 0.60 ml were removed after 0, 1, 2, and 3 minutes of nebulization or sonication. All samples were centrifuged at 10,000 x g for five minutes at room temperature. Protein concentration was estimated by transferring 60 ul of the supernatant portion of each sample to 3 ml aliquots of Bradford (Bradford, M.M. [1976]. Analytical Biochemistry 72: 218-254.) reagent and measuring the absorbtion at 595 nm.

Measurement of Temperature:

The temperature of each sample was measured at 0, 1, 2, and 3 minutes of nebulization and sonication.

Results:

Protein Release: The nebulized samples reached A_{595} peaks of 0.75 for yeast and 0.25 for *E. coli*. The sonicated samples gave A_{595} values of about 0.1 for both samples at all of the time points.

Temperature Change: The temperature of the BioNebulized samples decreased during the course of the experiments from a beginning temperature of around 20°C to a final temperature of 10°C for yeast and 15°C for *E. coli*. The temperature of the sonicated samples increased rapidly from 20°C to a final temperature of 50°C for yeast and 45°C for *E. coli*.

Conclusions:

The BioNeb System was much more effective in breaking both yeast and bacterial cells as determined by the release of protein. BioNebulization produced a cooling effect whereas sonication produced significant amounts of heat.

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TechTalk V

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Cyanobacteria (*Synechocystis 6803*)

Disruption Parameters:

The parameters that affect the amount of disruption are the supply gas (N_2 or He) pressure and the viscosity of the medium.

Medium:

10 mM Tris-Cl pH 7.8
5 mM EDTA
5 mM $MgCl_2$
1 mg/ml lysozyme
4 ug/ml DNase

Pressure:

The operating pressure range for disruption of cyanobacteria is approximately 100 psi.

Protocol:

1. Fresh or frozen cells were centrifuged at 5,000 x g for 10 minutes. The resulting pellet was resuspended in nebulization medium at a ratio of 100 grams packed cells to 250 ml medium. The resulting chlorophyll concentration of these preparations was 2.0 mg/ml.
2. Samples were passed through the BioNeb System three times at 100 psi He.
3. Samples were centrifuged to remove all green membrane fragments.
4. Disruption was monitored by reading the A_{620} of the supernatant and pellet. This gave a measure of the concentration of a soluble blue pigment protein which was released from disrupted cells.

Results:

Greater than 95% disruption was consistently obtained using the BioNeb System with the above protocol. Sonication typically yields about 45% disruption.(Dr. David Krogmann, personal communication) Sonication also causes significant heating of the sample. The above protocol takes about 90 minutes including cleanup and reassembly of the System.

Operation Notes:

Precooling the BioNeb system is not necessary because the very process of bionebulization causes a cooling of the solution which aids in stabilizing proteins. Temperature reductions of 10° C have been observed. The superior efficiency of disruption means that only half as much sample need be used resulting in significant savings of time and material.

The BioNeb Cell Disruption System cleans easily with soap and water. Ethanol can also be used to clean the unit. **DO NOT USE ACETONE.** The unit is completely autoclavable when sterilization is necessary, however, autoclaving will greatly reduce the life of the unit.

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TechTalk VI

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Carrot Cell Suspension Culture (*Daucus carota L.*)

2.0 g fresh weight of cells from log phase suspension cultures are resuspended in the following nebulization buffer on ice:

24mM Tris-MES pH 7.0
10 mM KCL
10 mM NaCl
15 mM $MgCl_2$
10 mM 2-mercaptoethanol
0.4 molal sorbitol
20% glycerol

Pressure:

The operating pressure range for obtaining intact nuclei from carrot cells is 80-120 psi He.

Protocol:

1. The nebulization sphere was brought into contact with the nozzle cap and the ball height adjustment disk was rotated counterclockwise to a nebulization deflector setting of 40 on the calibration disk.
2. Using the single pass batch mode, the sample was passed through the BioNeb System twice at 120 psi He gas pressure followed by two passes at 80 psi He gas pressure.
3. The nebulized sample was filtered through two layers of nylon mesh (40-50 um mesh) to remove unbroken cells and centrifuged at 35 x g for 5 minutes.
4. The pellet was resuspended in 0.5 ml of nebulization buffer and layered on top of a 15% / 25% / 50% (v/v/v) (2ml / 4ml / 2ml) Percoll gradient made in nebulization buffer plus sorbitol to give a final concentration of 0.4 molal sorbitol.
5. The gradient was centrifuged at 7,650 x g in a Sorvall SS-34 fixed angle rotor for 30 minutes at 4^0C.
6. The nuclei were collected at the 25% / 50% gradient interface.

Results:

This procedure yielded an average of 1×10^5 nuclei from 2.0 g fresh weight of cells (approximately 2×10^6 cells)

Marker enzyme assays for cytochrome c oxidase and NADH-dependent cytochrome c reductase indicated that there was little or no contamination of the nuclei fraction with mitochrondria or endoplasmic reticulum respectively.

The purified nuclei actively incorporated [8,5-^{3}H]-GTP into RNA.

Critical Parameters For Isolation Of Nuclei:

1. Early log phase (2 day) cultures gave better results than late log phase (4 day) cultures.
2. Increasing the cell concentration resulted in lower yields.
3. For suspension culture cells, the optimal pH was 7.0

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TechTalk VII

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Algal Cells: ***Chlamydomonas reinhardtii*** cu15(-) cell wall deficient strain: mating type$^-$

Cells were grown at 25°C in dim light (approximately 250 lux) in TAP (20 mM Tris-acetate, pH 7.2; 1 mM potassium phosphate, pH 7.0) to a cell density of 2 x 10^6 cells/ml.

Nebulization Medium:

0.3 M sorbitol
50 mM Hepes KOH, pH 7.2
2 mM EDTA, pH 8.0
1 mM $MgCl_2$

Harvested and washed cells were resuspended in nebulization buffer to yield a cell density of 7.5 x 10^7 cells/ml.

Pressure:

The operating pressure range for obtaining chloroplasts from *Chlamydomonas reinhardtii* cu15(-) is 20-22 psi N_2.

Protocol:

1. Cells were cooled on ice for three minutes prior to nebulization.
2. The BioNeb System was cooled to 4°C prior to use.
3. Cells were passed through the BioNeb System for one cycle at 20-22 psi N_2.
4. The sample was centrifuged at 5,000 rpm in a Sorvall HB4 rotor at 4°C for 20 minutes.
5. The resulting pellet was resuspended in 3 ml of cold nebulization medium and layered on a Percoll step gradient (10 ml 75% Percoll and 10 ml 45% Percoll in 0.3 M sorbitol, 50 nM TrisCl pH 8.0 and 10 mM $MgCl_2$) The gradient was centrifuged at 7,000 rpm in an HB4 rotor at 4°C for 20 minutes.
6. Chloroplasts were collected from the 75% / 45% interface.
7. Chloroplast yields were determined by comparing the chlorophyll concentrations of the nebulized samples with those of the gradient-purified chloroplasts.

Results:

An average yield of 35% was obtained under the described conditions.

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TechTalk VIII

Application Data for the Glas-Col BioNeb® Cell Disruption System

Organism:

Dinoflagellates (*Symbiodinium microadriaticum*)

Disruption Parameters:

The parameters that affect the amount of disruption are the supply gas type (N_2 or He), gas pressure, gas flow rate and the viscosity of the medium.

Volume:

The minimum volume of cells that can be disrupted at one time is 5 ml. There is no upper volume limit if the BioNebulizer is used in the open drain, single cycle mode or the open drain, continuous mode with auxiliary feed and receiver vessels. However, longer inlet and outlet tubes are required which can be supplied from in-house materials.

Recommended Parameters:

Gas type: N_2
Gas pressure: 90 psi.
Gas flow rate: approximately 6.5 liters/minute

Protocol:

1. Cells were suspended in 50 mM sodium phosphate buffer (pH 7.8) at a cell concentration of 4×10^4 cells/ml.
2. Samples were passed through the BioNebulizer at 90psi N_2 for three minutes in the continous mode, with stirring.
3. Treated samples were observed microscopically to determine the percent of disruption.

Results:

The percentage of disruption with the above protocol was 91% as determined by microscopic observation.

Operation Notes:

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