Your Application and Cell Success

CONSTANT SYSTEMS Ltd.
Cell Disruption Solutions

Constant Cell Disrupter Systems are extremely versatile and have been used for the following range of applications:

Cell Disruption, Protein & DNA Extraction, Enzyme Release, DNA Manipulation, Selective Breakage, Single Cell Isolation, Tissue Disruption

Individual organisms and samples processed by Constant Cell Disrupter Systems are too numerous to mention them all, however the following lists examples:

Algae	Disruption of anabaena variables
Animal Cells	Selective breakage of chicken sperm
Bacteria	Good enzyme activity from recombinant e.coli. High protein recovery from mycobacterium smegmatis
Fungi (inc. yeasts)	High specific activity of enzyme from Aspergillus nidulans. 99% protein release from saccharomyces cerevisiae. 99% Recombinant protein release from pichia pastoris
Mammalian Cells and tissue	Disruption of cellular and intracellular plasma membranes from horse liver Isolation of viable single cells from calf spleen and lymph node
Parasites	Breakage of cytomegalovirus in nucleus of human cells
Plant Cells & Tissue	Pectin extraction from banana fruit. Strawberry leaf DNA extraction
Viruses	Isolation of membrane antigens from toxoplasma gondi. Separation of nucleus and membrane in amniotic cells
Environmental Samples	Extraction of DNA from soil bacteria

E.coli Disruption & Guidance List

Constant Systems Cell Disrupters experience with E.coli is vast and breakage depends on the cell culture conditions, length of growth cycle and pressure utilised. We state the following as a guide to users for pressure compared to breakage with E.coli:

Release of DNA -
Below 15,000 psi (1000 Bar)

Cytoplasmic Protein -
15,000 psi - 20,000 psi (1350 Bar)

Inclusion Bodies -
20,000 psi - 27,000 psi (1900 Bar)

Membrane Protein -
27,000 psi - 35,000 psi (2400 Bar)

Other Species

Please find below a list of some of the other more common species of yeast with pressure vs. breakage:

- Candida Cloacae - 40 Kpsi
- Candida Albicans - 25 - 40 Kpsi
- Candida Utilis - 15 - 40 Kpsi
- Hansenula Polymorpha - 25 - 40 Kpsi
- Pichia Augusta - 40 Kpsi
- Pichia Capsulatus - 40 Kpsi
- Pichia Picorna - 25 - 35 Kpsi
- Pichia Pastoris - 35 - 40 Kpsi
- Kluyveromyces Fragilis - 05 - 40 Kpsi
- Rhodotorula Glutinis - 40 Kpsi
- Schizosaccharomyces Pombe - 15 - 40 Kpsi
- Saccharomyces Cerevisiae - 27 - 35 Kpsi

Examples of High Pressure Cell Disruption

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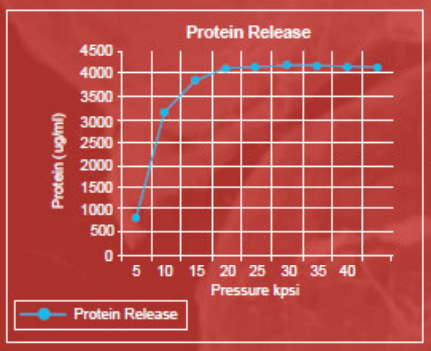
1.The effect of pressure on the cell disruption of Esherichia coli expressing soluble protein X

Completed at an independent and confidential customer site, 2003.

Preparation:

Cell paste (87g WCW) resuspended in 870ml 20mM TrisHCl/5 mM EDTA, pH 8.0. 40ml of the suspension was passed through Constant Cell Disruption Systems (Z Plus Series) once with the following disruption pressures: 10, 17, 20, 24, 27, 30, 33 and 35 kPSI respectively. Samples were centrifuged at 8000 rpm for 20 minutes at 4°C. Determined protein released with coomassie assay. Thereafter repeat passes of the same sample were passed through the cell disrupter to determine if multiple passes improved efficiency in protein yield between passes.

Results 1 - Protein Release:



Pressure (kpsi)	Protein (ug/ml)
0	414
10	3221
17	3838
20	4106
24	4162
27	4261
30	4247
33	4240
35	4236

Number of Passes	Protein (ug/ml)
1	4261
2	4840
3	4884
4	4488

For this particular strain of e.coli 27kpsi was the optimal pressure

Colony Forming Units (cfu) per ml of sample
OD_{600} 1 = 0.4g/L dry cell weight
OD_{600} 1 = 10^9 cfu/ml, therefore OD_{600} = 5.40 in 10L
Resuspended cell paste in 1L = OD_{600} = 54 which gives 5.4 x 10^{10} cfu/ml. Use this value to calculate percentage disruption at 27kpsi.
Percentage cell disruption at 27kpsi = 99.98% disruption with 1 pass through the cell disrupter. 99.98% breakage would leave 0.02% intact = 1.1 x cfu/ml 10^7
For the above experiment cell paste was suspended in 1:10w/v with lysis buffer. The experiment was repeated with a 1:30w/v dilution of cell paste with lysis buffer.

Sample	10^{-2}	10^{-4}	10^{-6}	CFU/ml
Non-disrupted	TNC	TNC	80	8.0 x 10^8
1 Pass	TNC	688	6	8.0 x 10^7
2 Pass	TNC	15	0	8.0 x 10^6

TNC: Too numerous to count
Fermentation 20: OD600 at harvest = 5.51/10L therefore 1L = 55, which gives 5.5 x 10^{10} cfu/ml in the non disrupted cells

Cell Disruption following 1 pass through the cell disrupter = 99.87%. 99.87% breakage would leave 0.13% intact = 7.2 x 10^7 cfu/ml
Cell Disruption following 2 pass through cell disrupter = 99.997%. 99.997% breakage would leave 0.003% intact = 1.7 x 10^6 cfu/ml.

2.The effect of pressure on the cell disruption of Saccharomyces Cerevisiae expressing protein X

Completed at an independent and confidential customer site, 2003.

Preparation:

Cell pellet was resuspended in chilled 20mM Tris/1 mM EDTA, pH 7.98. The required lysis buffer was calculated as follows: (46.82% w/v20%w/v) x 233ml = 545ml. The resuspended cells were mixed for 30 minutes at 6°C. pH and conductivity of resuspended paste; 6.98 at 12.2°C and 1.448mS/cm at 13.8°C. OD_{600} of resuspended cells; 76.

Method 1:

40ML of the resuspended cells were passed through a Constant Systems disrupter once at the following pressures; 8, 10, 15, 20, 25 and 27kpsi.

Results 1:

The efficiency of cell breakage was determined by the following method: Total Protein - 1ml of aliquots were centrifuged at 13,000rpm for 30 minutes. The supernatant containing the souble proteins was removed and total protein was determined in this fraction.

Pressure	Protein mg/ml
Non Disrupted	0.88
8	2.20
10	2.89
15	4.16
20	4.34
25	4.41
27	4.61

Viable Cell Count - 1ml of the cell lysate was added to 9ml of sterilised PBS (10 fold dilution). The following procedure was used to carry out serial dilutions:

100ul of each sample was spread onto agar plates. Plates were incubated for 39 hrs at 22.5°C. Only those dilution factores that gave between 30 to 600 colonies were counted and used in the calculation for determining number of viable cells per ml. Colony forming units (cfu) of sample = average number of colonies x 10/dilution factor.

Dilution 1:10	Sample Type and Volume	Volume of PBS (ml)
10^{-1}	1ml of neat sample	9
10^{-2}	0.1 ml of 10^{-1}	9
10^{-3}	0.1 ml of 10^{-2}	9
10^{-4}	0.1 ml of 10^{-3}	9
10^{-5}	0.1 ml of 10^{-4}	9
10^{-6}	0.1 ml of 10^{-5}	9

Percentage cell disruption after 1 pass through the cell disrupter.

Sample	% Cell Disruption
Non Disrupted	0
8	52%
10	68%
15	73%
20	87%
25	96%
27	98.9%