A New Bench Top System for Sample Preparation Using Pressure Cycling Technology (PCT)

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Abstract

Sample preparation is often one of the most significant bottlenecks in limiting rapid discoveries in such diverse fields as agriculture, environment, biotechnology, and drug development. To this end, a Pressure Cycling Technology-based Sample Preparation System (PCT SPS) was developed by Pressure BioSciences, Inc. (formerly Boston Biomedica, Inc.) and introduced to the market in September 2002. This System uses a floor model instrument (Barocycler™ NEP2017) and single-use, disposable processing tubes (PULSE™ Tubes). The NEP2017, primarily an R&D tool for the centralized laboratory, has helped generate important discoveries in genomics and proteomics.

We now introduce a smaller, portable, and more affordable bench top instrument (Barocycler NEP3229). Designed for use by researchers in their own laboratory, the NEP3229 can process three samples simultaneously in PULSE Tubes, compared to six with the NEP2017. Both instruments control molecules by applying cycles of low and ultra-high hydrostatic pressure to samples (between ambient and 35,000 PSI, or 235 MPa) – thereby disrupting tissues, cells, and cellular structures and releasing their contents. Data show that the Barocycler NEP3229 and NEP2017 models are comparable to other standard laboratory techniques (such as mortar and pestle grinding, bead beating, and sonication) in the quality and quantity of released biomolecules. However, the PCT SPS offers greater versatility, reproducibility, and safety.

Extraction Using Pressure Cycling Technology Sample Preparation System (PCT SPS)

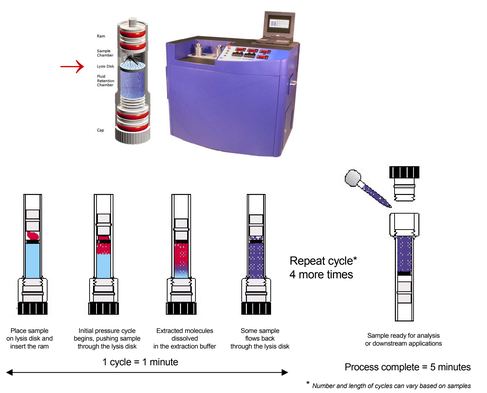


Fig. 1. The PCT Sample Preparation System includes an instrument, the Barocycler NEP2017 shown above or NEP3229 (Fig. 4), and specially designed PULSE Tubes. An illustration of the PCT extraction process is shown. → points to the lysis disk.

The Barocycler instrument can raise pressure from ambient to 35 kpsi (235 MPa) in less than 3 seconds and ramp down pressure from its maximum to ambient in a fraction of a second. Up to six (NEP2017) or three (NEP3229) PULSE Tubes can be processed simultaneously. The process temperature can be controlled using an external circulating water bath, allowing experiments to be performed between 4 and 37°C.

The FT500 PULSE Tubes can hold 20 – 500 mg of solid sample or 1.2 – 1.5 mL of liquid sample (PULSE Tubes that can process smaller and larger quantities are currently being developed). The sealed PULSE Tubes are immersed in pressurization fluid in the reaction chamber(s) of the Barocycler instrument and are then subjected to cycled pressure. The number of cycles, the pressure levels, and the temperature can be varied based on the material being processed. The entire extraction process takes place inside a sealed PULSE Tube, offering added safety and reduced opportunity for sample cross contamination.

Applications of PCT Extraction in Genomics and Proteomics

Using the PCT SPS (in Barocycler NEP2017), high quality total RNA and proteins were extracted from 20–500 mg of animal tissue including those known to contain high amounts of RNase and those that were high in lipid content, such as rat liver, brain, spleen, and tail.

Example 1: RNA Expression Profile of Rat Brain Analyzed on a Microarray

- 200 mg fresh frozen rat brain was extracted in 1.1 mL TRI Reagent (MRC) using standard PCT conditions, i.e. 35 kpsi or 235 MPa, five 1 min cycles at 4°C. A positive control sample was obtained using mortar-pestle grinding on dry ice.
- Crude lysate was clarified by centrifugation. Total RNA was purified using the TRI Reagent protocol, examined by formaldehyde agarose gel and quantified by OD_{260}.
- 1 µg of each purified total RNA was examined using the SuperArray microarray GEArray™ Q-series protocol on a MM-013N membrane - Neuroscience-1 Ion Channel & Transporter series (SuperArray Bioscience Corp, Frederick, MD). Chemiluminescent signals were detected by CCD camera.

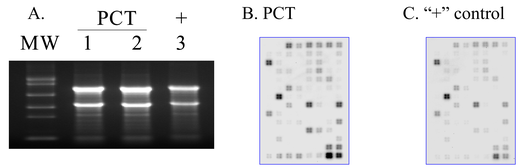


Fig. 2. RNA extracted from rat brain examined by formaldehyde agarose gel (A) and DNA microarray analysis using PCT SPS (B) and mortar-pestle (C) processed samples. Similar RNA expression patterns were obtained from samples processed using PCT and mortar-pestle grinding methods.

Example 2: Total Protein Extracted from Rat Liver Analyzed by 1D and 2D Gel Electrophoresis

- 200 mg fresh frozen rat liver was extracted in three different buffers (1D gel) and 100 mM Tris, 0.15M KCl, 1 nM PMSF, 1 mM DTT, and Sigma protease inhibitor cocktail (Cat. No. P8340) pH 8.0 (2D gel), using the standard PCT procedure. A positive control sample was obtained using mortar-pestle grinding on dry ice.

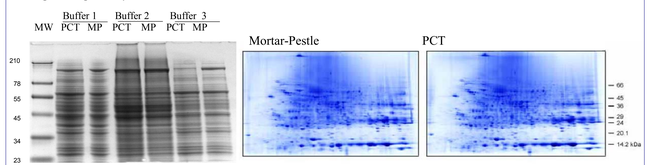


Fig. 3. Proteins extracted from rat liver examined using 1D SDS PAGE and 2DGE. Similar protein patterns were obtained by both the PCT and mortar pestle extraction methods.

Table 1 shows a comprehensive list of samples processed by PCT SPS (NEP2017) and various methods of downstream analyses applied to the released biomolecules.

Table 1. Samples Processed by PCT

Sample Group	Examples	Molecules Extracted[1,2]	Applications
Animal tissues	Soft: liver, brain, pancreas, kidney, lung	DNA, RNA, Proteins	PCR, RT-PCR, ELISA, SDS-PAGE, Western blot
	Hard: heart, skeletal muscle, breast tumor, aorta	DNA, RNA	PCR, RT-PCR
Plant tissues	Soft: corn sprout, leaves (corn, tomato, juniper, wheat), grape skin	DNA, RNA, Proteins	PCR, RT-PCR, SDS-PAGE
	Hard: stem, pine needle, grape seed	DNA	PCR
Insects, small organisms	Mosquito, *C. elegans*	DNA, RNA, protein	PCR, RT-PCR, SDS-PAGE
Blood	Blood, blood spot	DNA	PCR
Microbes	Yeast	DNA, RNA, Proteins	PCR, RT-PCR, SDS-PAGE
	Mycobacteria sp., bacteria/spores, soil	DNA, RNA	PCR

[1]Not every example has been tested for RNA or proteins with RT-PCR, WB or ELISA.
[2]Comparable yields were generally achieved in these experiments by comparing to either one or two conventional methods.

New Bench Top Barocycler

Pressure BioSciences now offers a bench top option, the Barocycler NEP3229 (Fig.4), for the PCT SPS. The NEP3229 has an external chiller hook-up, automatic fill and purge valves, and a key pad for a programmable microprocessor to set various PCT parameters. The NEP3229 is a lightweight, portable, and affordable instrument designed to fit on a laboratory bench top, inside a biological safety cabinet, or on the shelf of a cold room. This instrument can ramp up pressure from atmospheric pressure to 35 kpsi (235 MPa) in about 3 seconds and ramp down pressure from its maximum to ambient in a fraction of a second. Up to three PULSE Tubes can be processed simultaneously.

Barocycler™ NEP3229 - New

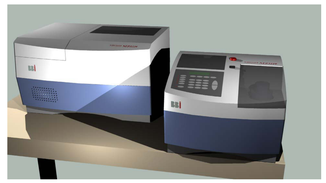
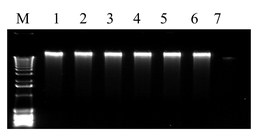


Fig. 4. New bench top Barocycler.

Here we show representative data from Corn Sprouts, Rat Liver, and Zebrafish comparing the Barocycler NEP2017 to the Barocycler NEP3229 and to conventional preparative methods for the release of DNA, RNA, or protein. The results are compared to those obtained from extraction methods using grinding by mortar and pestle or bead beating.

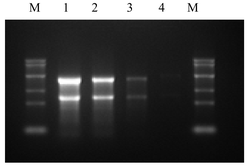
Fig. 5. DNA Released from Corn Sprouts



Lane	Method	Instrument
1, 2	PCT	NEP 2017
3, 4	PCT	NEP 3229
5, 6	Mortar & Pestle	
7	Negative	

200 mg samples were processed in 4M GTC, 1% NP40, RT PCT: 5 cycles (0.5'↑, 0.5'↓), 35 kpsi (235 MPa)

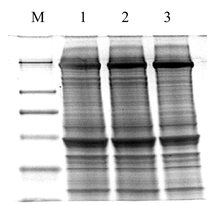
Fig. 6. RNA Released from Rat Liver



Lane	Method	Instrument
1	PCT	NEP 2017
2	PCT	NEP 3229
3	Bead Beating	
4	Negative	

200 mg samples were processed in 4M GTC, 1% NP40, RT PCT: 5 cycles (0.5'↑, 0.5' ↓), 35 kpsi (235 MPa)

Fig. 7. Protein Released from Zebrafish



Lane	Method	Instrument
1	PCT	NEP 2017
2	PCT	NEP 3229
3	Mortar & Pestle	
	Negative	

Whole fish were processed in 0.1 M Tris, 0.15 M KCl, 1 nM PMSF, protease inhibitor cocktail, RT. PCT: 5 cycles (0.5'↑, 0.5' ↓), 35 kpsi (235 MPa)

Table 2. Validation of Barocycler NEP3229 and compared to standard M/P grinding method.

EXTRACTION TARGET		METHOD	
		BAROCYCLER NEP3229	MORTAR-PESTLE
Total DNA	Avg. (µg)	167	122
	Std. Div (µg)	23.2	14.7
	CV (%)	13.9	12.0
Total RNA	Avg. (µg)	111	166
	Std. Div (µg)	14.8	24.7
	CV (%)	13.3	14.9
Total Protein	Avg. (mg)	16.0	13.4
	Std. Div (mg)	2.4	3.8
	CV (%)	15.2	27.9

In this validation study, 200 mg rat liver samples were used. Twenty four (24) duplicated samples were processed using both of the PCT SPS and mortar-pestle grinding methods.

On going validation study shows that the performance of the new bench top Barocycler (NEP3229) is similar to the floor model (NEP2017). The reproducibility of the PCT SPS is comparable to the current manual methods.

Discussion

The PCT SPS uses cycles of pressure between ambient and up to 35,000 psi (235 MPa) to extract DNA, RNA, small molecules, and proteins from a wide range of solid tissues or cell suspensions. The PCT SPS consists of either a floor model instrument (the Barocycler NEP2017) or the new bench top model (the Barocycler NEP3229).

The NEP2017 was designed to be used in a centralized laboratory and comes fully equipped with a circulating water bath and a computer to control PCT parameters. The NEP3229 was designed for use in individual laboratories and comes equipped with hook-up for a circulating water bath and with a micro-processor to set PCT parameters. The NEP3229 will be offered with a water bath (optional), and laboratory cart (optional), so that scientists may configure the PCT SPS to best meet their specific sample preparation requirements.

Our data indicate that whether configured with a Barocycler NEP2017 or NEP3229, the PCT SPS yields high quality and quantities of DNA, RNA, and protein from a wide variety of sample types. Furthermore, both configurations of the PCT SPS yield DNA, RNA and proteins comparable to conventional sample preparation methods. Regardless of which Barocycler model is used, the PCT SPS offers the additional, important features of increased safety, containment, reproducibility, and versatility, as well as reduced cross contamination, when compared to other current methods.

The PCT SPS is available with either a:

- Floor Model Barocycler NEP2017 Instrument
- Bench Top Model Barocycler NEP3229 Instrument

Comparable quality and yields of nucleic acids and proteins as released by conventional sample preparation methods may be obtained with either Barocycler model. Compared to these other methods, the PCT SPS is:

- Safer
- Faster
- Gentler
- More Powerful
- More Versatile
- More Reproducible

Please check the Pressure BioSciences booth during the GSAC for additional product information.

Acknowledgements:

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