

Pressure BioSciences Launches New FT500-ND PULSE Tube at The 22nd Symposium of The Protein Society

Introducing the FT500-ND PULSE Tube



The Newest Addition to the Pressure Cycling Technology (PCT) Family of Products

Unlike the FT500 PULSE Tube, the FT500-ND does not have a Lysis Disk. It was designed for processing solutions, suspensions, or complex matrices – such as soil – that do not require passage through a Lysis Disk for partial homogenization. A Blue Ram is first set into place using the provided PULSE Tube Tool. The sample and processing buffer are then added through the Cap end of the PULSE Tube and the Sample Chamber is closed by inserting the Blue Cap. Since there is no Lysis Disk, greater variation in both sample amount and processing buffer volume is possible than with the FT500 (researchers should consult specific applications for the use of the FT500-ND). Like the FT500, the FT500-ND transmits the power of PCT (pressure) from the Barocycler instrument to the sample. The assembled FT500-ND is placed in the pressure chamber of the Barocycler, PCT conditions are selected, and pressure cycling begins. During the PCT process, the Ram compresses the sample and processing buffer against the inside of the Blue Cap, resulting in pressure being transmitted to the sample. When one PCT cycle has finished, the Ram partially retracts, as pressure is released. This process is repeated for the requisite number of pressure cycles for a particular application. The combination of rapid pressure changes, chemistry, and other bio-physical mechanisms, together with the new diskless FT500-ND, make this new PULSE Tube ideal for processing bacteria, complex matrices, extracting mitochondria from cells, or for accelerating trypsin digestion and other enzymatic reactions under pressure.

Features & Benefits

- Versatile Range of Sample Sizes
- Versatile Range of Buffer Volumes
- Process Solutions & Suspensions
- Extract Mitochondria from Cells
- Extract Biomolecules from Soil Organisms

Application Focus

Mitochondria (Cell)-PrEP Isolation of Mitochondria from Cell Cultures for Proteomic Analysis by Pressure Cycling Technology

Introduction

Proteomic profiling of mitochondria has the potential to provide insights into mitochondrial functions associated with aging, various metabolic states, and diseases such as cancer, diabetes and cardiovascular disease. Rapid and reproducible isolation of intact mitochondria is crucial for efficient enrichment and subsequent proteomic analysis of low-abundance mitochondrial proteins. Here we describe a system for the isolation of intact mitochondria from rat PC12 cells using pressure cycling technology (PCT).

Materials and Methods

Mitochondria-enriched fractions were prepared from rat PC12 cells grown in suspension. Cells were pelleted by centrifugation and suspended in Mitochondrial Isolation Buffer (10 mM sucrose, 200 mM mannitol, 5 mM HEPES, 1 mM EGTA, pH adjusted to 7.4 with KOH and supplemented with 1 mg/mL fatty acid-free bovine serum albumin) [4]. The suspension was split into four aliquots, which were then processed using one of 4 sets of conditions to disrupt the cells: 1) Atmospheric pressure only ("0" kpsi); 2) 30 sec at 5 kpsi followed by 20 sec at atmospheric pressure, repeated for 15 cycles; 3) 30 sec at 15 kpsi followed by 20 sec at atmospheric pressure, repeated for 15 cycles; 4) 30 sec at 25 kpsi followed by 20 sec at atmospheric pressure, repeated for 15 cycles. After cell disruption by PCT, samples were centrifuged at low speed (900 × g) to pellet nuclei, large cellular debris and remaining intact cells. The resulting pellet (pellet 1) was saved and the supernatant was transferred to a fresh tube and centrifuged at high speed (13,000 × g) to separate the mitochondria (pellet 2) from the soluble cytosolic proteins. Pellets 1 and 2 were then washed with BSA-free buffer prior to analysis. Aliquots of pellet 1, pellet 2 and supernatant were separated by SDS-PAGE and were either stained with Coomassie Blue dye for total protein visualization (Figure 1), or were transferred to Immobilon-P (Millipore) for Western blot analysis (Figure 2). To confirm that the mitochondria-enriched fractions contain intact mitochondria, blots were probed with 3 antibodies (Abcam) that recognize proteins, localized to distinct mitochondrial compartments.

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CALENDAR OF EVENTS

IBC'S 13TH ANNUAL DDT

BOSTON, MA
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7TH ANNUAL WORLD HUPO

AMSTERDAM, NETHERLANDS
AUGUST 16-20, 2008

5TH INTERNATIONAL HPBB

LA JOLLA, CA
SEPTEMBER 15-19, 2008

BIOMARKER DISCOVERY SUMMIT

PHILADELPHIA, PA
SEPTEMBER 29-OCTOBER 1, 2008

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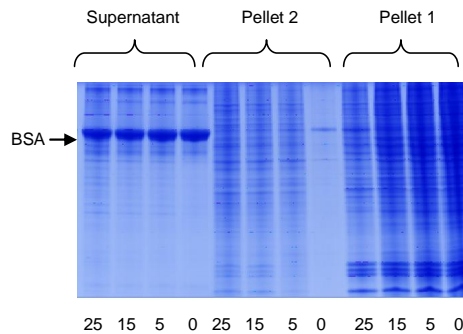


Figure 1. Coomassie blue-stained gel of pellet 1, pellet 2 and supernatant fractions from PC12 cells processed at the indicated pressure (0-25 kpsi). The BSA band in the supernatant is present due to the BSA added to the isolation buffer. Proteins were separated on an 8-16% Tris-HCl Criterion gel (BioRad).

Results and Discussion

The results shown in Figures 1 and 2 confirm that in the absence of PCT (0 kpsi control fractions) all mitochondrial markers are contained in the intact cells in pellet 1. Under initial PCT conditions, cells begin to lyse, and as pressure increases, more cells lyse and release mitochondria, which are then recovered in pellet 2. It is expected that if a significantly large number of mitochondria are ruptured, HSP60 would be detected in the supernatant fraction. The absence of strong mitochondrial protein signals in the cytosolic supernatant, especially soluble HSP60, supports the conclusion that the PCT protocol is gentle enough to lyse the cells while keeping the bulk of the mitochondria intact.

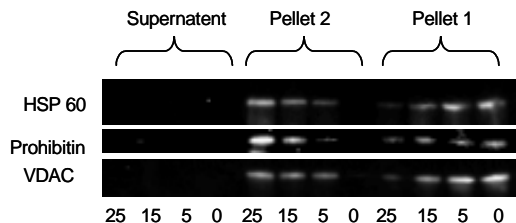


Figure 2. Western blots of pellet 1, pellet 2 and supernatant fractions from PC12 cells, pressure cycled at the indicated pressure. VDAC/Porin is a marker of the mitochondrial outer membrane; Prohibitin is a marker of the mitochondrial inner membrane; and HSP60 is a marker of the mitochondrial inner matrix space. The presence of all 3 proteins in the mitochondrial fraction (pellet 2) indicates that this fraction contains intact mitochondria.

These results show that PCT can be an effective method for the isolation of mitochondria from cultured cells. It is possible to optimize PCT to release mitochondria from other cells types and tissues. In addition, the stringency of the pressure cycling conditions can be modified based on the requirements of downstream applications. For example, PCT conditions can be adjusted to extract a smaller number of mitochondria under relatively gentle pressure for functional studies, or a larger number of mitochondria using more intense pressure cycling for proteomic analyses.

PBI in the News

Pressure Cycling Technology (PCT) Results in a Higher Yield of Proteins and in the Extraction of Proteins Not Previously Identified in Cancer, Stroke, and IVF Studies

PBI announced that scientists from the New York University ("NYU") School of Medicine and the Brooklyn Hospital Center presented results recently on advances they made in the areas of breast and colon cancer, stroke, and in vitro fertilization ("IVF"). The results were generated in studies using PBI's patented pressure cycling technology ("PCT") and patent-pending PCT-dependent ProteoSolve-LRS protein extraction kit. The presentations were made at the 2nd Annual Advances in Biomolecular Engineering: Protein Design Symposium, sponsored by the New York Academy of Sciences.

Dr. Paul H. Pevsner, NYU School of Medicine (Department of Pharmacology) and principle investigator for the research studies, commented: "PCT is critical to our work. This novel, cutting-edge technology consistently gives us rapid and reliable extraction of proteins from our important samples. In addition -- and this is very exciting -- PCT has given us the additional extraordinary benefits of not only greater protein yield, but the extraction of proteins not previously identified in these types of samples. Consequently, we believe that the use of PCT, more than the use of any extraction technology available today, could lead to the discovery of new biomarkers for cancer and stroke, which in turn could lead to the development of new diagnostics and therapeutics to combat these diseases."

Dr. Pevsner continued: "Many IVF live births result in twins, triplets, and higher order births. Unfortunately, fetuses and mothers of multiple and high-order multiple pregnancies have increased morbidity and mortality. To combat this, more single embryo transfers are needed. To this end, we have been interested in identifying biomarkers for embryo viability and competence. Using PCT and ProteoSolve-LRS, we identified two proteins in IVF growth media only seen in competent embryos. These proteins may prove to be biomarkers that can enhance selection and result in more live births from single embryo transfers. These are very exciting results."