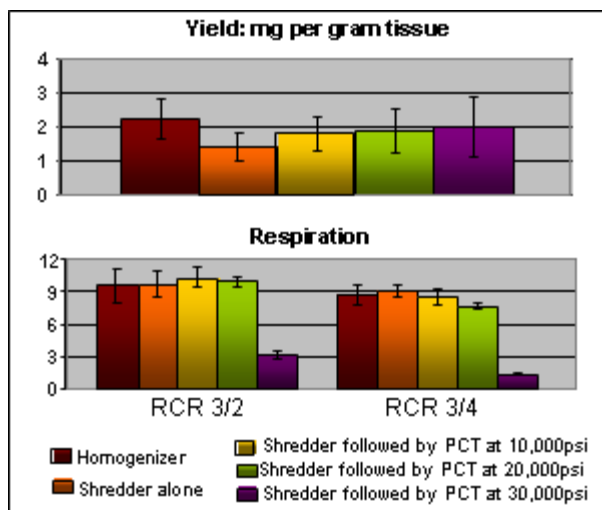


PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE

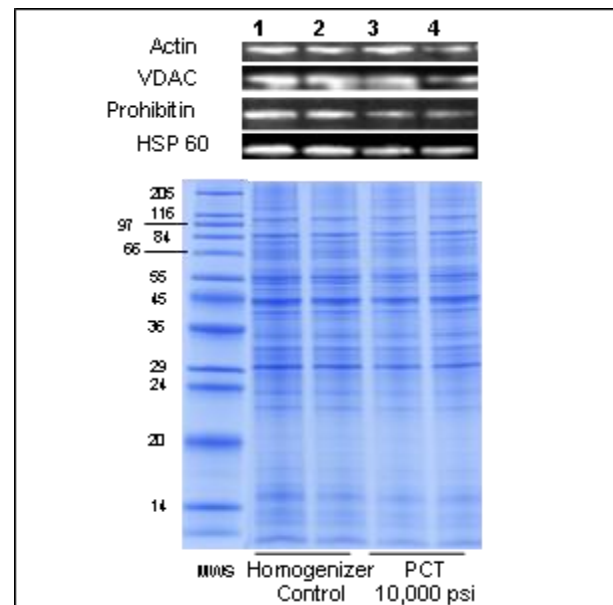
A Kit for the Isolation of Intact / Functional Mitochondria

Mitochondrial defects have been associated with a number of pathological conditions: muscle atrophy [1]; Parkinson's disease [2, 3]; atherosclerotic heart disease [4, 5]; cancer [6] and many others. Although it would be most relevant to assess mitochondrial functions *in situ* or *in vivo*, it is extremely difficult to separate primary mitochondrial effects from secondary effects because of the complexity of intact systems such as whole cells, tissues or organisms. Therefore, in order to better understand mitochondrial function and the interrelation of mitochondrial dysfunction with various diseases, it becomes necessary to extract mitochondria from tissue while maintaining them in an active and functional state.



Yield and Respiration of Skeletal Muscle Mitochondria

Mitochondria were extracted using a standard homogenizer method or *The PCT Shredder* with or without subsequent pressure cycling at 10,000, 20,000 or 30,000 psi. **Upper Panel:** More mitochondria are released by PCT after disruption with *The PCT Shredder* than by *The PCT Shredder* alone, with yields that approach the control method (Control, n=15; Shredder alone, n=19; 10,000psi, n=18; 20,000psi, n=3; 30,000psi, n=2). Final protein yield of the mitochondria-enriched preparations was measured by BCA assay. **Lower panel:** RCRs of mitochondria prepared using *The PCT Shredder* are comparable to homogenizer control. Mitochondria exposed to 30,000psi (n=2) are abnormal, while those treated at 10,000 psi (n=7) and 20,000 psi (n=3) are comparable to controls (homogenizer; n=5, Shredder alone; n=5).



Mitochondrial Preparations are Enriched in Markers for All Three Mitochondrial Compartments

Coomassie blue-stained SDS-PAGE gels and Western blots of mitochondrial samples isolated by homogenizer are essentially equivalent to the samples prepared using *The PCT Shredder* with pressure cycling at 10,000 psi. (VDAC, outer mitochondria membrane; Prohibitin, inner mitochondria membrane; HSP60, mitochondrial matrix).

Mitochondria isolation from solid tissue is usually carried out using labor-intensive homogenizer-based methods that require extensive operator experience and are prone to high variability between researchers. *THE PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE* uses a semi-automated method to generate a mitochondria enriched preparation using *The PCT Shredder* or *The SHREDDER SG3* for tissue homogenization. The rapid mechanical homogenization with either PBI shredder can be paired with an optional pressure cycling technology (PCT) step to further increase yield. Mitochondria prepared from freshly harvested tissue using the *PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE* are intact, normal and functional, as assessed by Western blotting, electron microscopy, Ca^{2+} uptake and respiration assays.

Features & Benefits:

- Easy-to-use Kit
- Obtain Intact, Normal, Functional Mitochondria
- Suitable for Western Blots, Electron Microscopy, Ca²⁺ uptake and Respiration Assays

Kit Contents

Product Name	Amount per Kit	Part Number
PBI Muscle Mitochondria Isolation Buffer (PBI MMIB) -10X stock solution	100 mL	030-1023-001
Protease Type XXIV	1 vial (50 mg)	030-1023-002
Enzyme Suspension Buffer	2 mL	030-1023-003
FT500-S PULSE Tubes, Caps, and Rams	36 each	030-1023-004
Microcentrifuge Tubes with Matching Plastic Pestles	12 each	030-1023-005

For Research Use Only. Not for Use in Diagnostic Procedures.

Kit Storage

Upon receipt, remove and store lyophilized enzyme at -20°C.
Store PCT MMIB and enzyme suspension buffer at 4°C.
Once reconstituted, aliquot enzyme and store at -20°C.
Thaw each aliquot only once.

Equipment Required But Not Provided:

1. *The PCT Shredder* or *The SHREDDER SG3*
2. Round Bottom Centrifuge Tubes (such as a Beckman 355642)
3. A Compatible Fixed-angle Rotor (such as a Beckman F0630)
4. A Compatible Refrigerated Centrifuge Capable of Reaching 14,000 RCF (14,000 g) (such as a Beckman Allegra X-22R)
5. Refrigerated Microfuge Capable of Reaching 14,000 RCF (14,000 g)
6. Fine Scissors and Tweezers for Mincing Tissue
7. Small Dishes, such as 60mm Petri, for Mincing Tissue
8. Plastic Transfer Pipets
9. (optional) Barocycler NEP 2320 or NEP 3229, with Circulating Water bath.

Note: The **PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE** works in conjunction and requires PBI's *The PCT Shredder* or *The SHREDDER SG3*. Increased yields may be obtained by an optional PCT treatment in PBI Barocyclers. Shredders and Barocycler are sold separately.

Application Note

AN-00020: *Isolation of Functional Mitochondria from Rat Skeletal Muscle Using The PCT Shredder with an Optional Pressure Cycling Technology (PCT) Processing Step*

References

- [1] Chabi et al., (2008) *Aging Cell*. 7(1):2-12
- [2] LeWitt. (2006) *J. Neural Trans. Suppl.* 71:113-22
- [3] Ogawa et al., (1992) *Neurology* 42(9):1702-1706
- [4] Ballinger. (2005) *Free Radic. Biol. Med.* 38(10):1278-1295
- [5] Madamanchi and Runge. (2007) *Circ Res.* 100(4):460-473
- [6] Hervouet et al., (2007) *Biochimie.* 89(9):1080-1088
- [7] *The SHREDDER SG3 User Manual*
- [8] *The PCT Shredder Manual*

Ordering Information

To order **PBI MITOCHONDRIA ISOLATION KIT: RAT MUSCLE** or other accessories, please contact your local Pressure BioSciences Sales Representative.

Or, please visit: <http://www.pressurebiosciences.com>

