MitoCheck® Mitochondria (Cell Culture) Isolation Kit

Item No. 502871

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
401064	Mitochondria (Cell Culture) Isolation Buffer A (5X)	2 vials/10 ml	4°C
401065	Mitochondria (Cell Culture) Isolation Buffer B (5X)	1 vial/12.5 ml	4°C
401135	Cell Permeabilization Reagent	1 vial/500 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's MitoCheck® Mitochondria (Cell Culture) Isolation Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A refrigerated centrifuge and rotor capable of 900 x g and 12,000 x g
2. 2 ml glass Dounce homogenizer
3. Microcentrifuge tubes
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Protein assay (*i.e.*, BCA, Bradford); *NOTE: Protein quantification assays are available for purchase from Cayman (Item Nos. 701780 and 760200)*
6. Membrane permeability assay (*e.g.*, Trypan Blue staining)

INTRODUCTION

Background

Isolated mitochondria provide a simple and biochemically relevant model to study mitochondrial biology in physiological and pathophysiological contexts.[1,2] They can be used to study electron transport chain enzymology or defects, measure energy metabolism, identify mutations in mitochondrial DNA (mtDNA) and mitochondrial tRNA, and compare cell type- or tissue-dependent mitochondrial defects. Several methods have been used to isolate mitochondria from cultured cells.[1,3] Cayman's MitoCheck® Mitochondria (Cell Culture) Isolation Kit isolates mitochondria from cultured cells using mechanical and chemical plasma membrane disruption and differential centrifugation in isotonic buffers.

About This Kit

Cayman's MitoCheck® Mitochondria (Cell Culture) Isolation Kit enables the isolation of functionally intact mitochondria from cultured cells in less than 60 minutes. Cells are initially resuspended in an isotonic buffer and subjected to mechanical disruption to liberate organelles. This is followed by sequential differential centrifugation to first remove nuclei and undisrupted cells and subsequently pellet mitochondria.

An optional digitonin cell permeabilization reagent is also provided to facilitate plasma membrane disruption, which may enable increased mitochondrial yields. Digitonin mediates selective disruption of cholesterol-rich membranes, resulting in plasma membrane permeabilization, while mitochondrial membranes, which are comparatively low in cholesterol, remain intact.[4]

This kit provides sufficient reagents to perform up to 60 isolations. Mitochondria isolated using this kit are functionally intact and may be used immediately for respirometry studies or other downstream application. Additional Cayman MitoCheck® assay kits may be used to characterize mitochondrial function (see Table 1, below).

Item No.	Item Name
700930	MitoCheck® Complex I Activity Assay Kit
700940	MitoCheck® Complex II Activity Assay Kit
700950	MitoCheck® Complex II/III Activity Assay Kit
700990	MitoCheck® Complex IV Activity Assay Kit
701000	MitoCheck® Complex V Activity Assay Kit
701040	MitoCheck® Citrate Synthase Activity Assay Kit

Table 1. Additional MitoCheck® assay kits available from Cayman

PRE-ISOLATION PREPARATION

Reagent Preparation

1. **Mitochondria (Cell Culture) Isolation Buffer A (5X) - (Item No. 401064)**

   Each vial contains 10 ml of concentrated Mitochondria (Cell Culture) Isolation Buffer A. Prepare 1.5 ml of Buffer A (1X) for each sample by diluting 300 µl of the concentrated buffer with 1,200 µl of ultrapure water. Store on ice until use. Use the diluted buffer within 24 hours of preparation. Do not freeze the concentrated or diluted buffer.

2. **Mitochondria (Cell Culture) Isolation Buffer B (5X)- (Item No. 401065)**

   This vial contains 12.5 ml of concentrated Mitochondria (Cell Culture) Isolation Buffer B. Prepare 1 ml of Buffer B (1X) for each sample by diluting 200 µl of the concentrated buffer with 800 µl of ultrapure water. Store on ice until use. Use the diluted buffer within 24 hours of preparation. Do not freeze the concentrated or diluted buffer.

3. **(Optional) Cell Permeabilization Reagent - (Item No. 401135)**

   This vial contains 500 µl of a 2% digitonin solution, which may enhance plasma membrane disruption. Thaw the vial at room temperature and vortex briefly, then store on ice until use. If not using all at once, prepare aliquots and freeze at -20°C. If precipitates form on thawing, heat the vial to 95°C and briefly vortex to redissolve components. It is strongly recommended that this reagent is titrated for each particular cell line (See **Cell Permeabilization Reagent Optimization**, page 9).

   Once the optimal digitonin concentration has been identified, prepare the Cell Permeabilization Reagent at 1X the desired final concentration using Buffer A (1X). Prepare 900 µl per sample and store on ice until use. Use the Cell Permeabilization Reagent (1X) solution within 24 hours of preparation.

Sample Preparation

It is recommended that 2-4 x 10^7 cells are used for each preparation. Example input and final yields expressed in terms of total protein (mg) are shown in Table 2, below.

Cell Line	Input Cell Count	Typical Mitochondria Yield (mg)
C2C12	2.0 x 10^7	~0.5
HEK293T	3.5 x 10^7	~0.5
SHSY-5Y	2.0 x 10^7	~0.5

Table 2. Example cell inputs and mitochondrial yields

Culture cells under conditions appropriate for the downstream application. Consideration should be given to the degree of confluency as some cell lines can undergo apoptosis as they become overgrown, which will compromise mitochondrial function.[5] Nutrient status and the presence of antibiotics can additionally negatively impact mitochondrial function.[6,7]

PROTOCOL

(Optional) Cell Permeabilization Reagent Optimization

While mechanical disruption may be sufficient to obtain optimal cellular disruption, the use of the optional Cell Permeabilization Reagent may improve the efficiency of this process. If using Cell Permeabilization Reagent, it is recommended that the user titrates the concentration of Cell Permeabilization Reagent with each cell line as excess digitonin can negatively impact mitochondrial function. Example titration data are shown in Figure 1 on page 10.

1. Resuspend cells in culture medium at ~5 x 10^6 cells/ml.
2. Prepare Cell Permeabilization Reagent working dilutions according to Table 3, below. Store on ice.
3. Combine 450 µl of cells with 50 µl of each ice-cold working dilution.
4. Incubate on ice for 5 minutes.
5. Perform Trypan Blue staining or a similar assay.
6. Visualize the cells under a microscope or imaging device and score the percentage of permeabilized cells.
7. Select the lowest concentration that gives >95% permeabilized cells.

Final %	Volume of Cell Permeabilization Reagent (µl)	Volume of Cell Culture Medium (µl)
0.02	20	180
0.01	10	190
0.003	3	197
0.002	2	198
0.001	1	199
0	0	200

Table 3. Preparation of Cell Permeabilization Reagent working dilutions

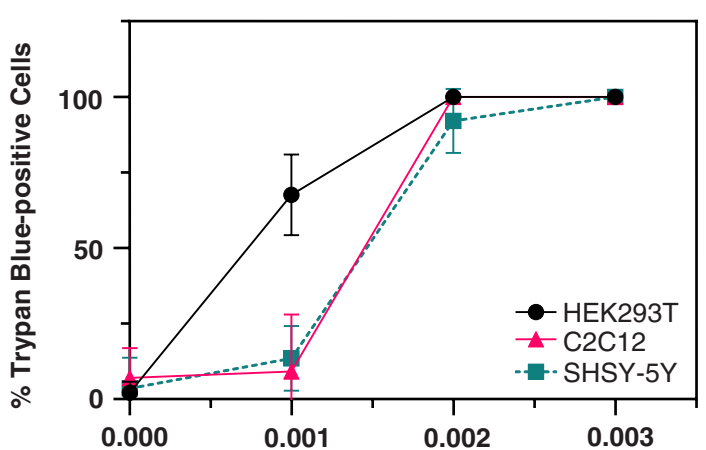

Figure 1. Cell Permeabilization Reagent titration. HEK293T, C2C12, and SHSY-5Y cells were incubated for 5 minutes with Cell Permeabilization Reagent, and the percentage of Trypan Blue-positive cells was scored.

Isolation Protocol

NOTES:

- *Perform all steps at 4°C.*
- *Ensure homogenizer is detergent-free.*
- *Pre-chill the homogenizer, buffers, and the optional Cell Permeabilization Reagent on ice.*

1. Pellet cells (2-4 x 10^7 cells per sample) *via* centrifugation at 250 x g for 5 minutes at 4°C.
2. Carefully remove medium and resuspend cells in 800 µl of Buffer A (1X) or Cell Permeabilization Reagent (1X).
3. Incubate cells on ice for 5 minutes.
4. Transfer the cell suspension to a pre-chilled Dounce homogenizer with a tight-fitting pestle. *Do not sonicate samples.*
5. Homogenize cells on ice. The number of strokes will vary depending on the instrument. Typically, 10-20 passes is sufficient to disrupt most cell lines.
   - (Optional) Transfer a 5 µl aliquot to a cover slip and view under a microscope to assess lysis efficiency.
6. Transfer the homogenate to a microcentrifuge tube.
7. Centrifuge at 900 x g for 5 minutes at 4°C.
8. Carefully remove the supernatant and transfer to a fresh microcentrifuge tube.
   - (Optional) If the remaining pellet appears similar in size to the initial cell pellet, resuspend in 500 µl of Buffer A (1X) and repeat steps 6-8. Combine both supernatants.
9. Centrifuge the supernatant at 12,000 x g for 15 minutes at 4°C.
10. Carefully remove the supernatant, which contains the cytosolic fraction, and resuspend the mitochondrial pellet with 800 µl of Buffer B (1X) *via* gentle pipetting.
11. Centrifuge at 12,000 x g for 15 minutes at 4°C.
12. Discard the supernatant. The pellet contains mitochondria.

Handling and Storage of Isolated Mitochondria

After isolation, gently resuspend the mitochondrial pellet in an appropriate volume of ice-cold Buffer B (1X), typically between 100 and 500 µl per preparation, depending on the expected yield and the requirements of subsequent experiments. Avoid vortexing or vigorous pipetting during this step to prevent mechanical damage to the mitochondrial membrane. *NOTE: Buffer B (1X) is protein-free and compatible with protein assays. Mitochondria resuspended in this buffer are functionally intact and suitable with downstream respirometric analyses.*

A typical preparation yields approximately 0.3–0.5 mg of mitochondrial protein (see Table 2, on page 8). The protein concentration should be determined using a BCA or Bradford protein assay to normalize mitochondrial input across experiments.

For Coupled Mitochondrial Respiration (*e.g.*, OCR or Membrane Potential Studies): Freshly isolated mitochondria should be kept on ice and used within two hours of isolation. Do not freeze. Freezing disrupts both the outer and inner membranes, resulting in loss of membrane potential and coupling efficiency. Use these preparations immediately for oxygen consumption rate (OCR), ATP synthesis, or membrane potential assays that require intact and energized mitochondria.

For Complex I-V Activity Assays: Flash freeze mitochondria, store at -80°C (if necessary), and thaw once prior to use. A single freeze-thaw cycle permeabilizes the outer membrane, allowing substrates and cofactors to access the inner membrane enzyme complexes. Frozen mitochondria are preferred for MitoCheck® Activity Assays as this treatment standardizes membrane permeability and improves assay reproducibility across samples.

ANALYSIS

Performance Characteristics

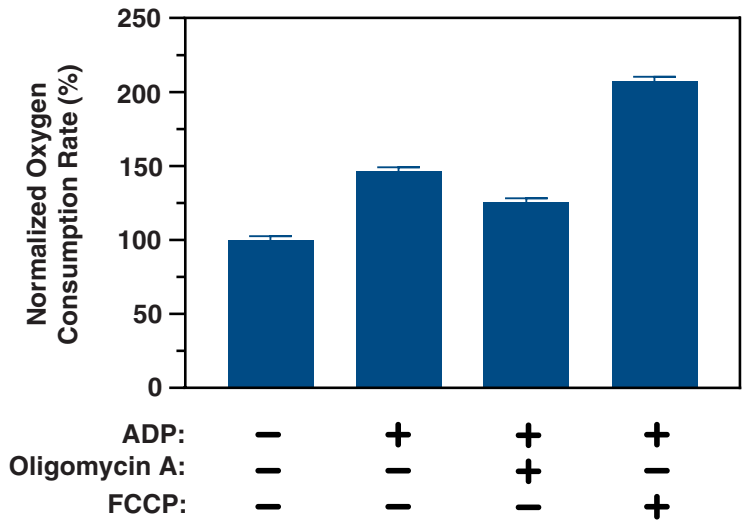

Figure 2. Oxygen consumption of isolated mitochondria. Mitochondria isolated from C2C12 cells (70 µg/well) using Cayman's Mitocheck® Mitochondria (Cell Culture) Isolation Kit were resuspended in respiration medium containing 2 mM malate, 10 mM glutamate, and a fluorescent oxygen-sensitive probe. Oxygen consumption increased upon addition of ADP, indicating state 3 respiration, and further stimulation was observed with FCCP, reflecting uncoupled respiration. Simultaneous addition of oligomycin A to ADP-treated mitochondria led to a decrease in oxygen consumption, consistent with inhibition of ATP synthase. Oxygen consumption rates were normalized to untreated mitochondria, which were set to 100%. ADP (Item Nos. 16778 | 21121), oligomycin A (Item No. 11342), and FCCP (Item No. 15218) are available for purchase from Cayman.

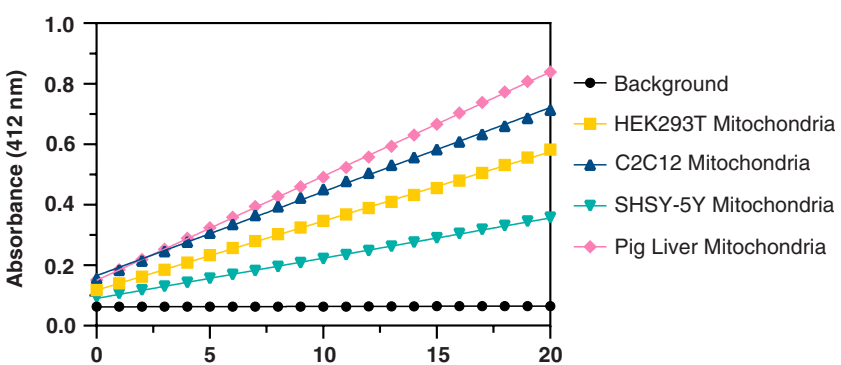

Figure 3. Citrate synthase activity of isolated mitochondria. Citrate synthase activity of various isolated mitochondria were assayed using Cayman's Mitocheck® Citrate Synthase Activity Assay Kit (Item No. 701040). Pig liver mitochondria (2.3 µg/well) were isolated using Cayman's Mitocheck® Mitochondria (Tissue) Isolation Kit (Item No. 701010). Mitochondria from HEK293T (0.39 µg/well), C2C12 (0.34 µg/well), and SHSY-5Y (0.25 µg/well) cells were isolated using this kit.

RESOURCES

Troubleshooting

Problem	Possible Causes	
Low mitochondrial yield	A. Low cell input B. Insufficient lysis	A. Increase number of cells B. Increase the number of strokes with homogenizer OR supplement with Cell Permeabilization Reagent before homogenization
Low rates of mitochondrial oxygen consumption, low mitochondrial membrane potential, or poor respiratory control ratio	Mitochondria have degraded	A. Assay mitochondria immediately after isolation if assay requires coupled mitochondria B. Ensure samples are maintained at 4°C throughout the isolation procedure C. Ensure glassware is detergent-free

References

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3. Bronfman, M., Loyola, G., and Koenig, C. S. Isolation of intact organelles by differential centrifugation of digitonin-treated hepatocytes using a table eppendorf centrifuge. *Anal. Biochem.* **255(2)**, 252-256 (1998).
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6. Krampe, B. and Al-Rubeai, M. Cell death in mammalian cell culture: Molecular mechanisms and cell line engineering strategies. *Cytotechnology* **62**, 175-188 (2010).
7. He, C., Eggelbusch, M., Huijts, J.Y., *et al.* The commonly used antibiotic streptomycin reduces protein synthesis and differentiation in cultured C2C12 myotubes. *Physiological Reports* **13(12)**, (2025).

Isolation Summary

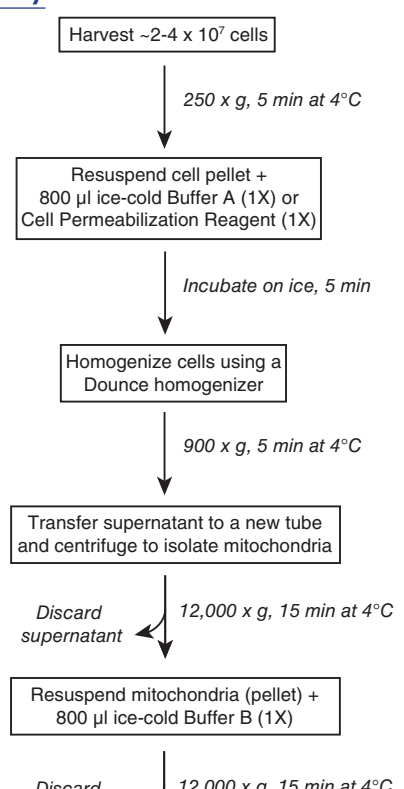

Figure 4. Mitochondria isolation summary

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.