ExKine™ Mitochondrion Extraction Kit (Tissue)

Item NO.	Product Name
KTP4004	ExKine™ Mitochondrion Extraction Kit (Tissue)

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

Version 201809

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INTRODUCTION

Background & Principle

Mitochondria is a double-membrane-bound organelle found in most eukaryotic cells. The function of mitochondria is to provide cellular energy. Moreover, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorder syndrome, heart failure and autism. Mitochondria may play an important role in these cellular processes.

The Mitochondria Isolation Kit enables rapid and crude isolation of intact mitochondria from animal tissues from both soft and hard tissues using differential centrifugation. This crude mitochondrial preparation is often enough for most applications, such as study of mitochondrial respiration, mitochondria membrane potential, apoptosis, mtDNA and mtRNA, and mitochondrial protein profiling etc.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 12 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

- Assay kit is intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

PRODUCT INFORMATION

Materials supplied and Storage conditions

Kit components	Quantity		Storage conditions
	50T	200T	
Lysis Buffer A (5×)	20 mL	80 mL	-20°C
Lysis Buffer B (5×)	20 mL	80 mL	-20°C
Storage Buffer	3 mL	10 mL	-20°C

Other supplies required, Not Supplied

- Microcentrifuge
- Pipettes and pipette tips
- Phosphate-buffered saline (PBS)
- Glass tissue homogenizer
- 0.25 mg/ml trypsin

Technical hints

- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Note: Perform all steps at 2–8 °C. Use precooled buffers and equipment. Ensure all the solutions are defrosted and homogeneous.

Reagent Preparation

Lysis Buffer A (5×) and Lysis Buffer B (5×): Aliquot and store unused solutions at -20° C. Defrost at 37°C to achieve a clear solution. Diluting with sterile, deionized water. Immediately before use, add protease inhibitors. Keep on ice while using.

Storage Buffer: Aliquot and store unused solutions at -20°C. Keep on ice while using.

Recommended procedures

A. For soft tissues (liver or brain)

1. Prepare a fresh tissue sample (obtained within one hour of sacrifice) and wash the sample twice with ice-cold PBS.
Note: Do not freeze.

2. Cut 100 mg of tissue into very small pieces and wash the sample once with 1 mL ice-cold Lysis Buffer A.

3. Add fresh 1mL ice-cold Lysis Buffer A and transfer to a pre-cooling Dounce homogenizer.

4. Homogenize the sample on ice (usually 10-20 strokes).

5. Transfer the homogenate to a new tube and centrifuge the sample at 600g for 5 minutes.
Note: For a more purified “heavy” mitochondrial fraction, this step can be changed to centrifuge at 1000 g for 5 minutes. The BSA (delipidated, final concentration 2mg/mL) can be added to the Lysis Buffer A to remove lipids, which may be present in the tissue.

6. Collect the supernatant in a new tube and centrifuge at 11,000 x g for 10 min at 4°C.
Note: To obtain a more purified fraction of mitochondria, with >50% reduction of lysosomal and peroxisomal contaminants, this step can be changed to centrifuge at 3000 g for 15 minutes. The supernatant is cytosol fraction.

7. Remove the supernatant and resuspend the pellet in 1 mL ice-cold Lysis Buffer A. Repeat steps 5 and 6.

8. Suspend the pellet (purified mitochondria) in Storage Buffer (40 μl per 100 mg tissue). Freeze and aliquot at -80°C until use.
Note: It is expected the protein concentration of the sample should be approximately

10-25 mg/mL.

B. For hard tissues (skeletal or heart muscle)

Note: For heart muscle, use Lysis Buffer A and for skeletal muscle use Lysis Buffer B. For other tissues, use Lysis Buffer A first.

1. Prepare a fresh tissue sample (obtained within one hour of sacrifice) and wash the sample twice with ice-cold PBS.
Note: Do not freeze.

2. Cut 100 mg of tissue into very small pieces. Centrifuge the sample at 600g for 30s, and then discard the supernatant.

3. Suspend the sample in 1mL 0.25 mg/mL trypsin in a new 2 mL Eppendorf tube.

4. Incubate on ice for 5-10min.
Note: The incubation time varies in different tissues.

5. Spin down the tissue for a few seconds in the centrifuge and remove the supernatant.

6. Wash the sample once with 1 mL ice-cold the appropriate Lysis buffer and spin down the tissue for a few seconds in the centrifuge.

7. Remove the supernatant and add fresh 1 mL ice-cold the appropriate Lysis buffer.

8. Transfer the sample to a pre-cooling Dounce homogenizer and homogenize the sample on ice (usually 20-30 strokes).

9. Transfer the homogenate to a new tube and centrifuge the sample at 600g for 5 minutes.
Note: For a more purified “heavy” mitochondrial fraction, this step can be changed to centrifuge at 1000 g for 5 minutes.

10. Collect the supernatant in a new tube and centrifuge at 11,000 x g for 10 min at 4°C.
Note: To obtain a more purified fraction of mitochondria, with >50% reduction of lysosomal and peroxisomal contaminants, this step can be changed to centrifuge at 3000 g for 15 minutes. The supernatant is cytosol fraction.

11. Remove the supernatant and resuspend the pellet in 1 mL ice-cold appropriate Lysis Buffer. Repeat steps 9 and 10.

12. Suspend the pellet (purified mitochondria) in Storage Buffer (40 μl per 100 mg tissue). Freeze and aliquot at -80°C until use.
Note: It is expected the protein concentration of the sample should be approximately 10-25 mg/mL.