Mitochondrial Extraction Kit

Cat No: SM0020

Size: 50T/100T

Storage: store at 4℃ within four weeks, store at -20℃ for long term preservation.

Components	SM0020 -50T	SM0020-100T
Lysis Buffer	50mL	100mL
Mito-Wash Buffer	25mL	50mL
Store Buffer	5mL	10mL

Description:

Mitochondrial Extraction Kit enables purify and isolation of intact mitochondria from animal tissue or cells. It is suitable for the preparation of mitochondrial in animal soft tissue, hard tissue and culture cells. It can be used to study apoptosis, signal transduction, metabolism and proteomics.

Protocal

1. Sample preparation

a. Tissue sample: Wash 100~200 mg fresh sample such as liver, brain, myocardium with PBS or saline. Wash blood and dry with filter paper, cut into pieces and put them into homogenizer. Add 1.0mL ice-cold Lysis Buffer, grind 20 times on ice bath.

b. Culture cell sample: digest cells, wash with PBS, centrifuge for 5~10min at 800g. 5×10^7 cells needed for every extraction. Add 1.0mL cold Lysis Buffer，transfer cell suspension into a small homogenizer, grind 30~40 times on ice bath.

2. Transfer the tissue or cell homogenate into centrifuge tube, 4℃, centrifuge for 5min at 1000g.
3. Transfer the supernatant into a new tube, 4℃, centrifuge for 5min at 1000g.
4. Transfer the supernatant into a new tube, 4℃, centrifuge for 10min at 12000g. The supernatant after centrifugation contain cytoplasm which can extract cytoplasm protein. Transfer supernatant to a new tube, the mitochondrial deposit at the bottom of tube.
5. Add 0.5mL Wash Buffer to precipitation resuspend the mitochondrial sediment, 4℃, centrifuge for 5min at 1000g.
6. Transfer the supernatant into a new tube, 4℃, centrifuge for 10min at 12000g. Discard supernatant. High purity mitochondria precipitate at the bottom of the tube.
7. Add 50-100μL Store Buffer or properly buffer to resuspension of mitochondrial sediment. Use immediately or store at -70℃.

Note

I. To obtain complete mitochondrial, whole process should operated in low temperature condition and operate quickly. Maintain the integrity of subcellular organelle while breaking cells . This is the most critical part of the preparation of mitochondria. CompareD with tissue pellet, it is difficult to break the wall of culture cells when using glass homogenizer, especially adherent cells. So choose small glass homogenizer to grind cells.

II. Use centrifugal force(g) to calculate centrifugal speed, different centrifuges can accurately calculate the centrifugal speed according centrifugal force.

III. Directly add buffer to lyse mitochondrial in Western Blot and 2D-gel electrophoresis.

$G = 1.11 \times (10\text{-}5) \times R \times [rpm]^2$

G:centrifugal force(g)

$[rpm]^2$: the square of rotate

R: Radius, cm

Related Products

P1020 1×PBS，PH7.2-7.4，0.01M

P1015 SDS-PAGE loading buffer,4×(with DTT)

SN0020 Nuclear Extraction Kit

Reference Published Paper

[1] Mengnan Zeng,1,Beibei Zhang,Benke Li,et al. Adenosine Attenuates LPS-Induced Cardiac Dysfunction by Inhibition of Mitochondrial Function via the ER Pathway.Evidence-Based Complementary and Alternative Medicine Volume. September 2018. (IF 2.064)

[2] Zongwen Liu,Zhenjiang Guo,Alan Chu,et al. High incidence of coding gene mutations in mitochondrial DNA in esophageal cancer. Molecular Medicine Reports. September 2017. (IF 1.922)