New

LIFE SCIENCE

Mitochondrial Isolation Kit

Mitochondrial Isolation Kit

1kit [M3527]

M3527 can be used to easily isolate mitochondria from cultured mammalian cells and tissue.
Isolated mitochondria can be used for downstream analyses such as Western blotting.

Kit Components

- **Mitochondrial Isolation Reagent A for approx. 30 samples**
- **Mitochondrial Isolation Reagent B for approx. 30 samples**
- **Mitochondrial Isolation Reagent C for approx. 30 samples**

Application: Isolation from Mouse-Derived Cells

1. Collect cells by centrifugation and remove the supernatant without disturbing the cell pellet. Use a cell scraper for detaching adherent cells.
2. Resuspend cells in Mitochondrial Isolation Reagent A and vortex for 5 seconds. Add 400 µL per 1 x 10^7 cells.
3. Incubate on ice for 2 minutes.
4. Add Mitochondrial Isolation Reagent B to the cell suspension and vortex for 5 seconds. Use 5 µL per 400 µL of suspension from step 2.
5. Incubate on ice for 5 minutes, vortexing once per minute.
6. Add Mitochondrial Isolation Reagent C and invert several times (do not vortex). Use a volume equal to the volume of Reagent A used in step 2.
7. Centrifuge at 700 x g for 10 minutes at 4°C.
8. Transfer the supernatant to a new tube and centrifuge at 12,000 x g for 15 minutes at 4°C.
9. Collect the supernatant (cytoplasmic fraction) and add 300 µL of Mitochondrial Isolation Reagent C to the pellet (mitochondria).
10. Centrifuge at 12,000 x g for 5 minutes at 4°C and remove the supernatant.
11. Use mitochondria (pellet) for downstream experiments.

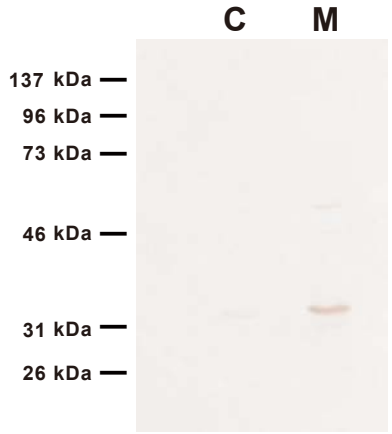

Figure 1.
Western blotting of M3527-isolated cytoplasmic fraction (C) and mitochondria (M), detected with VDAC1/2 antibody.
Mitochondrial proteins were extracted with RIPA Buffer.
M3527 allows for highly efficient purification of mitochondria

Mitochondrial Isolation Kit

Application: Isolation from Mouse Liver

1. Weigh 50-100 mg of mouse liver and wash with PBS(-).
2. Add 400 µL of Mitochondrial Isolation Reagent A and homogenize.
3. Incubate on ice for 2 minutes.
4. Add 5 µL of Mitochondrial Isolation Reagent B and vortex for 5 seconds.
5. Incubate on ice for 5 minutes, vortexing once per minute.
6. Add 400 µL of Mitochondrial Isolation Reagent C and invert several times (do not vortex).
7. Centrifuge at 700 x g for 10 minutes at 4°C.
8. Transfer the supernatant to a new tube and centrifuge at 12,000 x g for 15 minutes at 4°C.
9. Collect the supernatant (cytoplasmic fraction) and add 300 µL of Mitochondrial Isolation Reagent C to the pellet (mitochondria).
10. Centrifuge at 12,000 x g for 5 minutes at 4°C and remove supernatant.
11. Use mitochondria (pellet) for downstream experiments.

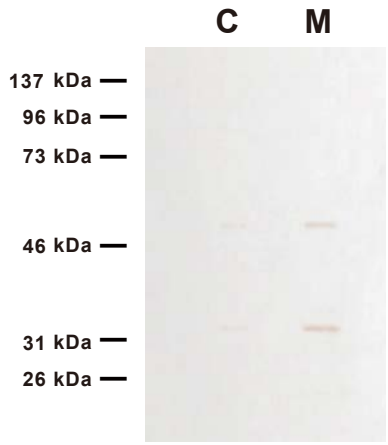

Figure 2.
Western blotting of M3527-isolated cytoplasmic fraction (C) and mitochondria (M), detected using VDAC1/2 antibody.
Mitochondrial proteins were extracted using RIPA Buffer.
M3527 allows for highly efficient purification of mitochondria from mouse liver.

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TCI AMERICA
Tel : 800-423-8616 / 503-283-1681
Fax : 888-520-1075 / 503-283-1987
E-mail : Sales-US@TCIchemicals.com

TCI EUROPE N.V.
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Fax : +32 (0)3 735 07 01
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Fax : +49 (0)6196 64053-01
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E-mail : Sales-UK@TCIchemicals.com

梯希爱(上海)化成工业发展有限公司
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Fax : 021-6712-1385
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E-mail : Sales-IN@TCIchemicals.com

TOKYO CHEMICAL INDUSTRY CO., LTD.
Tel : +81 (0)3-5640-8878
E-mail : globalbusiness@TCIchemicals.com