New

LIFE SCIENCE

Membrane Protein Extraction Kit

Membrane Protein Extraction Kit

1kit [M3842]

M3842 can be used to extract membrane proteins easily from cultured mammalian cells and tissue. The extracted proteins can be used directly in downstream applications such as Western blotting.

Kit Components

- **Permeabilization Buffer** for approx. 50 samples
- **Permeabilization Buffer Additive** for approx. 50 samples
- **Solubilization Buffer** for approx. 50 samples

‡ Dissolve Permeabilization Buffer Additive in Permeabilization Buffer before first use. Aliquot and store at −20°C.

Application: Extraction from Mouse-Derived Cells

1. Collect cells via centrifugation and wash twice with PBS(-). Use a cell scraper for detachment of adherent cells.
2. Resuspend cells in cold Permeabilization Buffer with Additive, adding 500 µL for every 5 x 10^6 cells.
3. Incubate at 4°C for 10 minutes with constant mixing.
4. Centrifuge at 16,000 x g for 15 minutes at 4°C. Transfer the supernatant (cytosolic fraction) to a new tube.
5. Resuspend the pellet in cold Solubilization Buffer. Use half the amount of buffer used in step 2.
6. Incubate at 4°C for 30 minutes with constant mixing.
7. Centrifuge at 16,000 x g for 15 minutes at 4°C. Transfer the supernatant (membrane fraction) to a new tube.
8. Use each fraction for downstream experiments.

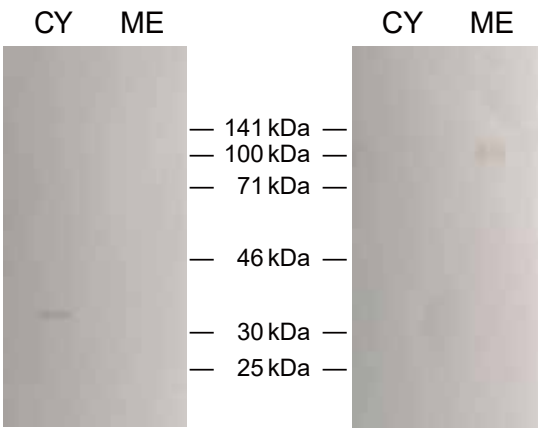

Figure 1.
Western blotting of a cytosolic (CY) and membrane (ME) fraction extracted with M3842 from mouse-derived cells, detected using GAPDH antibody (left) and ATP1A1 antibody (right).
Highly purified membrane proteins were obtained from mouse-derived cells.

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Application: Extraction from Mouse Heart

1. Weigh out 20 mg of mouse heart and wash with PBS(-).
2. Add 500 µL of cold Permeabilization Buffer with Additive and homogenize.
3. Incubate at 4˚C for 10 minutes with constant mixing.
4. Centrifuge at 16,000 x g for 15 minutes at 4˚C. Transfer the supernatant (cytosolic fraction) to a new tube.
5. Resuspend the pellet in 250 µL of cold Solubilization Buffer.
6. Incubate at 4˚C for 30 minutes with constant mixing.
7. Centrifuge at 16,000 x g for 15 minutes at 4˚C. Transfer the supernatant (membrane fraction) to a new tube.
8. Use each fraction for downstream experiments.

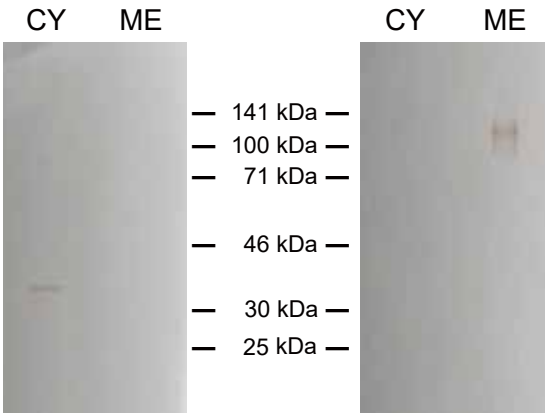

Figure 2.
Western blotting of a cytosolic (CY) and membrane (ME) fraction extracted with **M3842** from mouse heart, detected using GAPDH antibody (left) and ATP1A1 antibody (right).
Highly purified membrane proteins were obtained from mouse heart.

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