LIFE SCIENCE

TCI®

Cell Fractionation / Protein Extraction Reagents

Cell Fractionation Kits

Efficient organelle isolation is important for investigating protein localization and function. TCI offers reagent kits optimized for nuclear, cytoplasmic, and mitochondrial fractionation.

Mitochondrial Isolation Kit

Mitochondrial Isolation Kit

1kit [M3527]

M3527 can be used to easily isolate mitochondria from cultured mammalian cells and tissue.
The 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 of Mitochondrial Isolation Kit [M3527]

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 (cytosolic 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.

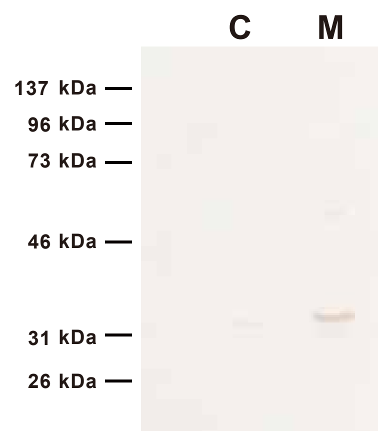


Figure 1.
Western blotting of M3527-isolated cytosolic fraction (C) and mitochondria (M), detected with VDAC1/2 antibody.
Mitochondrial proteins were extracted with RIPA Buffer.
M3527 allows for highly efficient purification of mitochondria

Nuclear / Cytoplasmic Fractionation Kit

Nuclear / Cytoplasmic Fractionation Kit

1kit [N1208]

N1208 can be used for convenient fractionation of nuclear and cytoplasmic proteins from cultured mammalian cells. By using the three reagents included, quick collection of each fraction can be achieved. The extracted proteins can be used directly in downstream applications such as Western blotting.

Kit Components

- **1X Cytoplasmic Extraction Buffer**
  for approx. 50 samples
- **20X Detergent Solution**
  for approx. 50 samples
- **1X Nuclear Extraction Buffer**
  for approx. 50 samples

Application of Nuclear / Cytoplasmic Fractionation Kit [N1208]

[For Cytoplasmic Extraction]

1. Collect cells and wash cells twice with ice cold PBS. Use a cell scraper for adherent cells.
2. Resuspend the cells gently in cold 1X Cytoplasmic Extraction Buffer. Add 400 µL of the buffer to 100 µL of the cell volume (~ 1 x 10^7 cells).
3. Incubate on ice for 10 minutes.
4. Add 20X Detergent solution to the cell suspension and vortex vigorously for 10 seconds. Use 25 µL of 20X Detergent Solution per 500 µL of the solution from step 3.
5. Centrifuge at 800 x g for 10 minutes at 4 °C.
6. Transfer the supernatant (cytoplasmic fraction) to a new tube for downstream experiments.

[For Nuclear Extraction]

7. Resuspend the pellet in cold 1X Nuclear Extraction Buffer. Add 100 µL of 1X Nuclear Extraction Buffer per 100 µL pellet.
8. Incubate the pellet solution on ice for 20 minutes, vortexing every 5 minutes.
9. Centrifuge at 15,000 x g for 10 minutes at 4 °C.
10. Transfer the supernatant (nuclear fraction) to a new tube for downstream experiments.

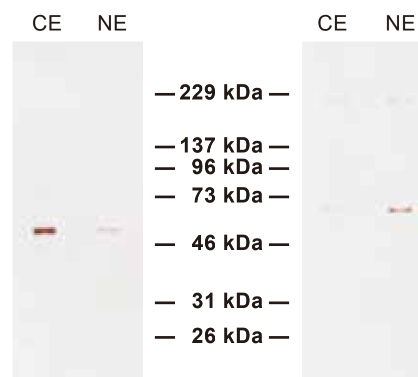


Figure 2.
Western blotting of cytoplasmic fraction (CE) and nuclear fraction (NE) extracted with N1208, detected using α-Tubulin antibody (left) and Lamin B1 antibody (right).
Excellent separation was achieved.

Ready-to-use Solutions for Protein Extraction

The first step in protein purification, lysing tissue or cells to extract protein, is crucial for properties and yield of the obtained protein. TCI offers optimal buffers for protein extraction from cultured cells, nervous tissue and microorganisms.

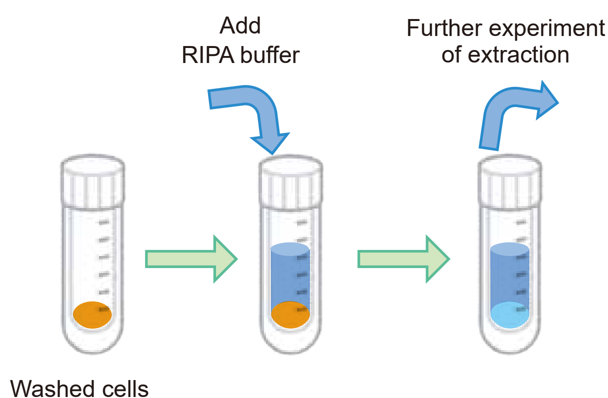
Nervous Tissue Protein Extraction Buffer	100mL [B6279]
RIPA Buffer (Ready-to-use) [for Protein extraction]	100mL [R0246]
E.coli / Yeast Protein Extraction Buffer	100mL [Y0021]

Advantages

- **Detergent-based ready-to-use solutions optimized for each research subject.**
- **The target protein can be obtained easily by suspending tissue or cells and centrifuging.**
- **Extracted proteins can be used directly in downstream analysis such as Western blotting.**

Application of RIPA Buffer [R0246]

1. Wash the cultured mammalian cells twice with PBS.
2. Resuspend cells in RIPA Buffer **[R0246].** Use 1 mL buffer per 0.5 - 5.0 × 10^7 cells.
3. Incubate on ice for 15 minutes.
4. Centrifuge the sample at 10,000 x g for 10 minutes at 4 °C.
5. Use supernatants for downstream experiments.



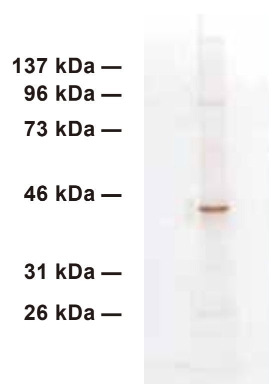


Figure 3.
Western blotting of extracted proteins using **R0246**.
β-Actin (42 kDa) can be extracted from mammalian cells efficiently.

Protease Inhibitor Cocktails

100×Protease Inhibitor Cocktail (EDTA free) 1vial [P2949]
100×Protease Inhibitor Cocktail 1vial [P2976]

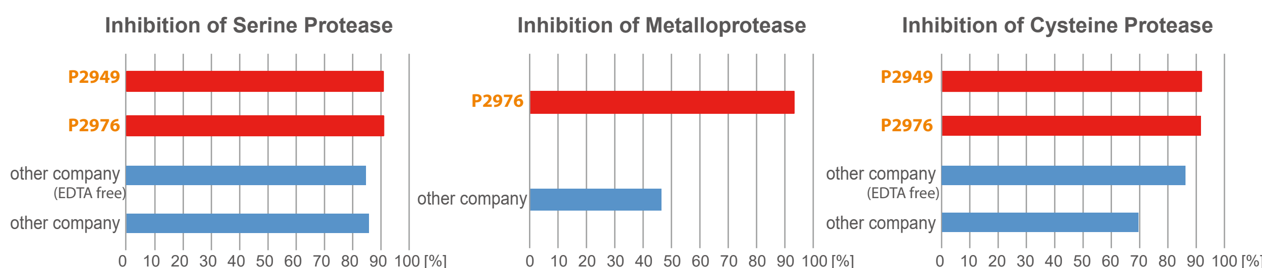
Advantages

- Can be used as a 100× stock solution by dissolving in 1 mL of deionized water.
- Protects protein samples from proteolysis by various proteases.
- P2949 does not contain EDTA, making it compatible with metal chelate affinity chromatography.

Components

Protease Inhibitor	Target Protease(s)	P2949	P2976
AEBSF	Serine protease	○	○
Aprotinin	Serine protease, Esterase	○	○
EDTA	Metalloprotease		○
E-64	Cysteine protease	○	○
Leupeptin	Cysteine protease, Trypsin-like protease	○	○

Protease Inhibition Tests



P2949 and P2976 show higher levels of inhibition against various proteases than other company's products.

Related Products

30% Acrylamide / Bis-acrylamide (29:1) 250mL [A3217]
30% Acrylamide / Bis-acrylamide (37.5:1) 250mL [A3218]
Bradford Assay Solution (Ready-to-use) [for Protein determination] 500mL [B5702]
2X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) 25mL [B5834]
4X SDS-PAGE Sample Buffer (2-Mercaptoethanol free) 20mL [B6104]
6X Sample Buffer (2-Mercaptoethanol free) 10mL [B6105]
2X SDS-PAGE Sample Buffer Phenol Red (2-Mercaptoethanol free) 25mL [B6110]
Coomassie Brilliant Blue G-250 (Ready-to-use Solution) [for Electrophorsis] 500mL [C3488]
DAB staining kit 1kit [D5909]
Pyrogallol Red (Ready-to-use Solution) [for Protein determination] 100mL [P2575]
Standard Solution of Albumin from Bovine Serum 5mL [T3796]

Ordering and Customer Service

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