YamayBio

Animal Membrane and Cytosol Protein Extraction Kit

QUICK START GUIDE

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

Animal Membrane and Cytosol Protein Extraction Kit

BF7475-10 10 Samples

BF7475-50 50 Samples

Storage:

- Cell Permeabilization Buffer and Cell Solubilization Buffer: Store at –20 °C. Avoid repeated freeze–thaw cycles.
- Cell Wash Solution: Store at 4 °C.

Component	Cat. No.	Volume BF7475-50	Volume BF7475-10	Storage
Cell Wash Buffer	BF7475-A	225 mL	45 mL	4°C
Cell Permeabilization Buffer	BF7475-B	50 mL	10 mL	–20 °C
Cell Solubilization Buffer	BF7475-C	25 mL	5 mL	–20 °C

Introduction

The Animal Membrane and Cytosol Protein Extraction Kit provides sufficient lysis and extraction reagents for the following sample numbers:

- Animal cells: 50 (or 10) pellets, each containing 5 × 10^6 cells
- Tissue samples: 25 (or 5) samples, each containing 20–40 mg of tissue

Additional Material Required

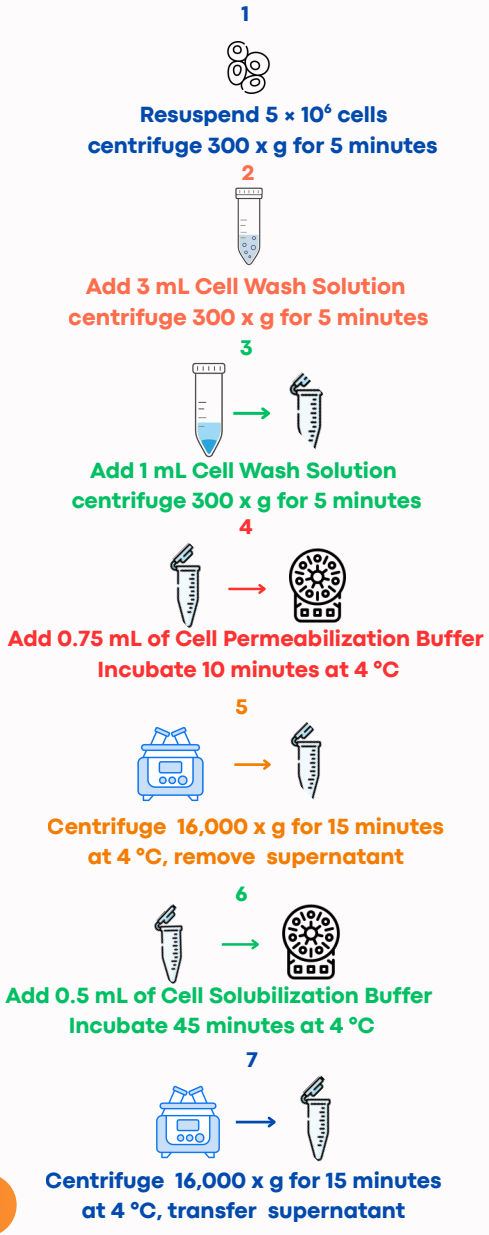
1. Protease inhibitors (e.g., Cat. No. BH5481).
2. Protease and phosphatase inhibitors (e.g., Cat. No. BH5483).
3. For tissue samples, a 2 mL Dounce tissue grinder (e.g., Kontes or Wheaton Tenbroeck) is required.

Important Product Information

1. Place thawed Cell Permeabilization Buffer and Cell Solubilization Buffer on ice.
2. Before use, add 1× protease inhibitor (e.g., Cat. No. BH5481) or 1× protease and phosphatase inhibitor (e.g., Cat. No. BH5483).
3. Aliquot Cell Permeabilization Buffer and Cell Solubilization Buffer to avoid repeated freeze–thaw cycles.
4. Select the appropriate protocol according to the sample type.

Procedure for Different Sample Types

Protocol 1: Adherent Animal Cells



1. Resuspend **5 × 10^6 cells** in growth medium by scraping cells from the culture surface. **Centrifuge** at **300 × *g* for 5 minutes**.

2. Wash the pellet with **3 mL Cell Wash Solution** and **centrifuge at 300 × *g* for 5 minutes**.

3. Carefully remove and discard the supernatant. Resuspend cells in **1 mL Cell Wash Solution**, transfer to a 1.5 mL centrifuge tube. **Centrifuge at 300 × *g* for 5 minutes** and discard supernatant.

4. Add **0.75 mL of Cell Permeabilization Buffer** to the cell pellet. Vortex briefly and **incubate at 4 °C for 10 minutes** with constant mixing.

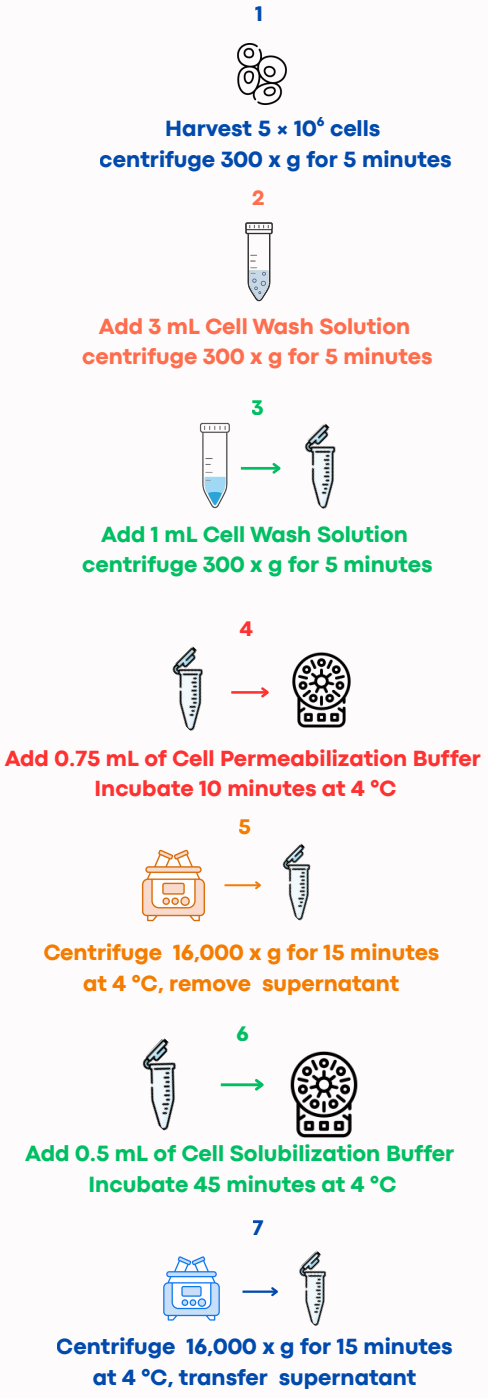
5. **Centrifuge at 16,000 × *g*** for **15 minutes at 4 °C**. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.

6. Add **0.5 mL Cell Solubilization Buffer** to the pellet and resuspend by pipetting up and down. **Incubate tubes at 4 °C for 45 minutes** with constant mixing.

7. **Centrifuge tubes at 16,000 × *g* for 15 minutes at 4 °C**. Transfer the supernatant containing solubilized membrane and membrane-associated proteins to a new tube.

8. Proceed to downstream applications or store aliquots at -80 °C for future use.

Protocol 2: Suspension Animal Cells



1. Harvest **5 × 10^6 cells** by **centrifugation at 300 × *g* for 5 minutes**.

2. Resuspend the pellet in **3 mL Cell Wash Solution** and **centrifuge at 300 × *g* for 5 minutes**. Carefully remove and discard the supernatant.

3. Resuspend the cells in **1 mL Cell Wash Solution** and transfer to a 1.5 mL centrifuge tube. **Centrifuge at 300 × *g* for 5 minutes** and discard supernatant.

4. Add **0.75 mL Cell Permeabilization Buffer** to the pellet. Vortex briefly to obtain a homogeneous suspension. **Incubate 10 min at 4 °C** with constant mixing.

5. **Centrifuge at 16,000 × *g* for 15 minutes**. Carefully transfer the supernatant (cytosolic proteins) to a new pre-chilled tube.

6. Add **0.5 mL Cell Solubilization Buffer** to the pellet and resuspend by pipetting up and down. **Incubate 45 min at 4 °C** with constant mixing.

7. **Centrifuge at 16,000 × *g* for 15 min at 4 °C.** Transfer the supernatant (solubilized membrane and membrane-associated proteins) to a new tube.

8. Proceed immediately to downstream applications, or aliquot and store at –80 °C. Avoid repeated freeze–thaw cycles.

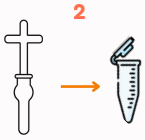
Protocol 3: Animal Tissue

1

20-40mg tissue
Add 4mL of Cell Wash Solution

1. Place **20-40 mg of tissue** in a 5 mL microcentrifuge tube. Add **4 mL of Cell Wash Solution**, vortex briefly, and discard the wash. Repeat if needed.

2



Add 1 mL Cell Permeabilization Buffer

2. Transfer tissue to a 2 mL tissue grinder and mince with scissors. Add **1 mL Cell Permeabilization Buffer** and homogenize on ice until an even suspension is obtained.

3

Add 1 mL Cell Permeabilization Buffer Incubate 10 minutes at 4 °C

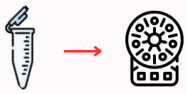
3. Add an additional **1 mL Cell Permeabilization Buffer** and transfer the homogenate to a new tube. **Incubate at 4°C for 10 minutes** with constant mixing.

4

Centrifuge 16,000 x g for 15 minutes at 4 °C, remove supernatant

4. **Centrifuge at 16,000 × *g* for 15 minutes at 4 °C** to pellet permeabilized cells. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.

5



Add 1mL Cell Solubilization Buffer Incubate 45 minutes at 4 °C

5. Resuspend the pellet in **1 mL Cell Solubilization Buffer.** Pipette up and down to obtain a homogeneous suspension. **Incubate at 4 °C for 45 minutes** with constant mixing.

6

Centrifuge 16,000 x g for 15 minutes at 4 °C, transfer supernatant

6. **Centrifuge tubes at 16,000 × *g* for 15 minutes at 4 °C**. Transfer the supernatant containing solubilized membrane and membrane-associated proteins to a new tube.

7. Proceed immediately to downstream applications, or aliquot and store at –80 °C. Avoid repeated freeze–thaw cycles.

Note:

1. Extracted membrane proteins include plasma membrane, mitochondrial membranes, endoplasmic reticulum membranes, Golgi apparatus membranes, etc.

2. For Western Blot analysis of extracted membrane protein samples, after mixing with loading buffer, samples may be processed as follows:
   - Load directly without heating
   - Heat at 50 °C for 10 minutes before loading
   - Heat at 37 °C for 30 minutes before loading

3. Protein concentration may be determined using a BCA protein assay kit (e.g., Cat. No. BH5484).