YamayBio

Animal Membrane and Cytosol Protein Extraction Kit

QUICK START GUIDE

Research use only.
Not for 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 tissue

Additional Material Required

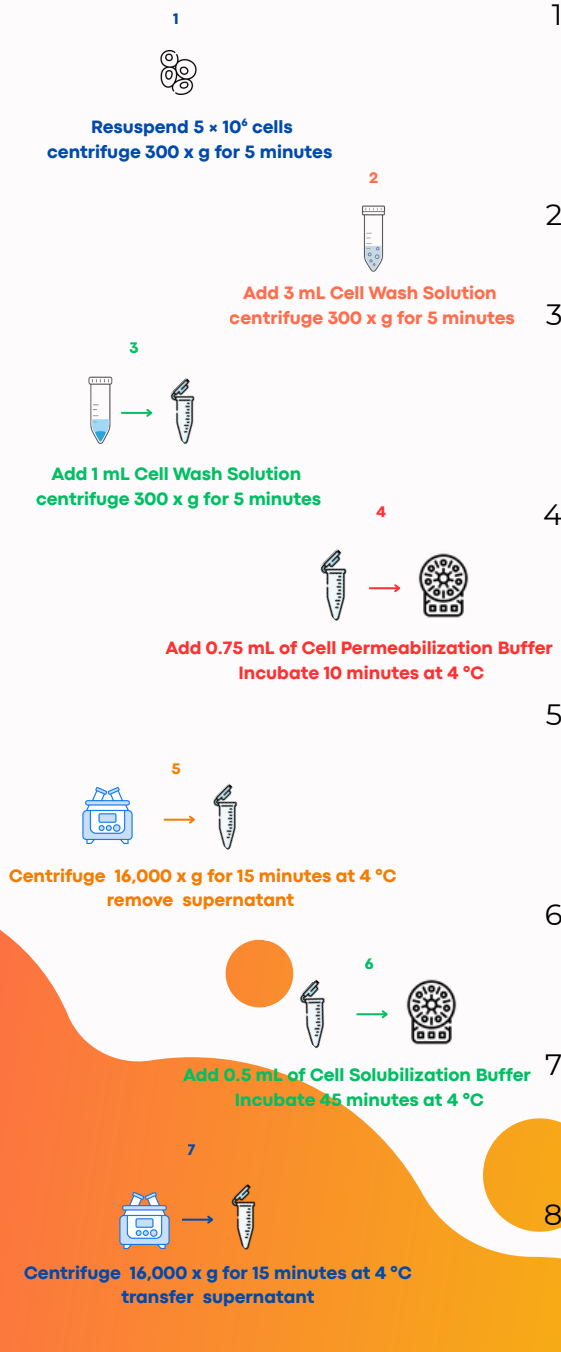
1. Protease inhibitors (e.g., Cat. No. BH5481).
2. Protease and phosphatase inhibitors (e.g., Cat. No. BH5483).
3. For tissues, a 2 mL Dounce Tissue Grinder (e.g., Kontes or Wheaton Tenbroeck) is required.

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. Store Cell Permeabilization Buffer and Cell Solubilization Buffer in aliquots to avoid repeated freeze–thaw cycles.
4. Select the appropriate protocol according to the sample type.

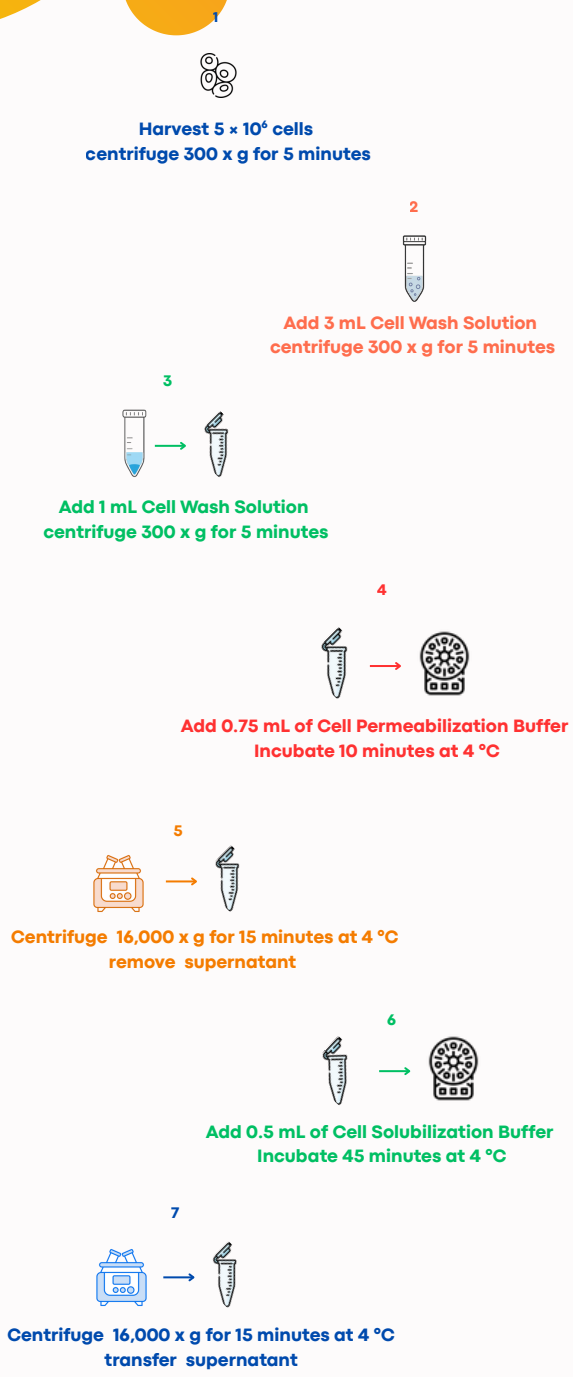
Procedure for Different Sample Types

Protocol 1: Adherent Animal Cells



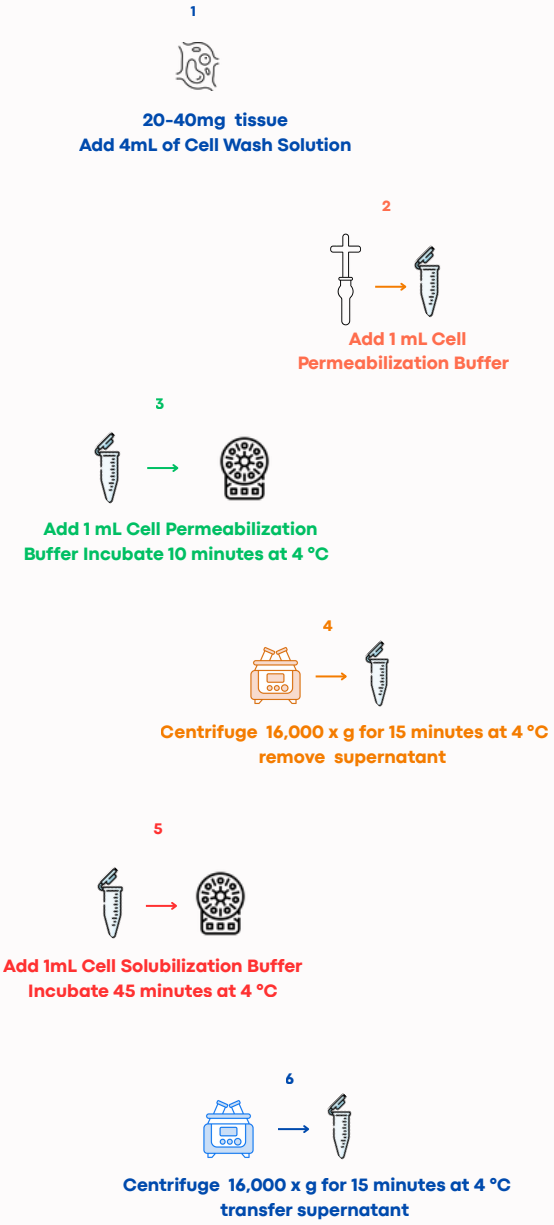
1. Resuspend **5 × 10^6 cells** in the growth media by scraping the cells off the surface of the plate with a cell scraper. **Centrifuge** harvested cell suspension at **300 × *g* for 5 minutes**.
2. Wash cell pellet with **3mL Cell Wash Solution** and **centrifuge at 300 × *g* for 5 minutes**.
3. Carefully remove and discard the supernatant. Resuspend the cells in **1mL Cell Wash Solution** and transfer to a 1.5mL centrifuge tube. **Centrifuge at 300 × *g* for 5 minutes** and discard supernatant.
4. Add **0.75mL of Cell Permeabilization Buffer** to the cell pellet. Vortex briefly to obtain a homogeneous cell suspension. **Incubate 10 minutes at 4°C** with constant mixing.
5. **Centrifuge** permeabilized cells for **15 minutes at 16,000 × *g* at 4°C**. Carefully remove the supernatant containing cytosolic proteins and transfer to a new tube.
6. Add **0.5mL 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 supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
8. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice 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 min**.
2. Resuspend the pellet in **3 mL Cell Wash Solution** and **centrifuge at 300 × *g* for 5 min**. Carefully remove and discard the supernatant.
3. Carefully remove and discard the supernatant. Resuspend the cells in **1mL Cell Wash Solution** and transfer to a 1.5mL 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 min**. 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 to downstream applications immediately, or aliquot cytosolic and membrane fractions and store at –80 °C. Avoid repeated freeze–thaw cycles.

Protocol 3: Animal Tissue



1. Place **20-40mg of tissue** in a 5mL microcentrifuge tube. Add **4mL of Cell Wash Solution** to the tissue, vortex briefly and discard wash (tissue can be cleaned once again).
2. Transfer to a 2mL tissue grinder and cut the tissue into small pieces with a pair of scissors. Add **1mL Cell Permeabilization Buffer** to the tissue and homogenize on ice until an even suspension is obtained.
3. Add **1mL Cell Permeabilization Buffer** and transfer homogenate to a new tube, **incubating for 10 minutes at 4°C** with constant mixing.
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. Resuspend the pellet in **1mL Cell Solubilization Buffer.** Pipette up and down to obtain a homogeneous suspension. **Incubate 45 minutes at 4°C** with constant mixing.
6. **Centrifuge tubes at 16,000 × *g* for 15 minutes at 4°C**. Transfer supernatant containing solubilized membrane and membrane-associated proteins to a new tube.
7. Proceed to downstream application. Immediately use cytosolic and membrane fractions stored on ice or store aliquots at -80°C for future use.

Note:

1. The extracted membrane proteins include plasma membrane, mitochondrial membranes, endoplasmic reticulum membranes, Golgi apparatus membranes, and so on.
2. When performing Western Blot experiments on extracted membrane protein samples, after pre-mixing with loading buffer, they can usually be processed in the following three ways:
- Load directly without heating.
- Heat the sample at 50°C for 10 minutes and then load.
- Heat the sample at 37°C for 30 minutes and then load.
3. The extracted protein samples can be quantified by using a BCA protein quantification kit (e.g., Cat. No. BH5484).