

 PRO-PREP™ Protein Extraction Solution [EDTA-free]
QUICK GUIDE

100 ml : 17081.1

1. KIT DESCRIPTION

PRO-PREP™ Protein Extraction Solution [EDTA free] is a ready-to-use reagent designed for rapid and convenient extraction of total protein from various cultured cells and tissues. The EDTA-free formulation contains four protease inhibitors to minimize protein degradation during the extraction process, with EDTA provided separately for optional use. The extracted proteins are suitable for various downstream applications, including Western blotting and protein quantification.

2. CHARACTERISTICS

- Easy and rapid total protein extraction from cultured cells and tissues within 20–30 minutes Rapid protein extraction within 20–30 minutes
- Contains protein stabilizing components to maintain protein stability during extraction
- Contains five protease inhibitors to minimize protein degradation without additional protease inhibitor treatment
- Remains unfrozen at -20°C, minimizing protein degradation caused by repeated freeze-thaw cycles
- Stable for more than 6 months when stored at -20°C
- Suitable for protein quantification and downstream applications such as Western blot and molecular weight analysis
- EDTA-free; EDTA provided separate

3. KIT CONTENTS AND STORAGE

Contents	17081.1
PRO-PREP™ Protein Extraction Solution	100 ml
EDTA Solution	0.2 ml

- Store at -20°C

4. CONSIDERATIONS BEFORE USE

- 1) Detergent Selection : Select an appropriate detergent according to the sample type and intended downstream application
- 2) Detergent Classification : Detergents are generally classified as ionic, non-ionic, or zwitterionic based on their ionic properties
- 3) Ionic Detergents : Ionic detergents strongly denature proteins and are suitable for applications such as Western blotting and molecular weight analysis.
- 4) Non-ionic Detergents : Non-ionic detergents such as Triton X-100 cause less protein denaturation and are commonly used when protein–protein interactions need to be preserved.
- 5) Zwitterionic Detergents : Zwitterionic detergents such as CHAPS have both positive and negative charges and generally cause less protein denaturation than ionic detergents.
- 6) Extraction Conditions : Detergent performance and micelle formation can be affected by pH, temperature, ionic strength, and other solution conditions. Appropriate extraction conditions should therefore be maintained for efficient protein extraction.

5. PROTEASE INHIBITOR

Protease inhibitor	Target	Working Concentration
PMSF	Serine and thiol proteases	1.0 mM (174 µg/mL)
Pepstain A	Acid proteases	1 µM (0.7 µg/mL)
Leupeptin	Serine proteases	1 µM (0.5 µg/mL)
Aprotinin	Serine and thiol proteases	0.1 µM (2.0 µg/mL)

6. PROTOCOL

6.1. Preparation of PRO-PREP™ Solution

[Optional : EDTA Addition]

If EDTA is required, add 0.1 ml of EDTA Solution per 100 ml of PRO-PREP™ Solution and mix thoroughly before use.

6.2. Sample Preparation

[Cultured Cell]

- 1) Prepare approximately 5 × 10⁶ cells.

Note : For adherent or suspension cells, collect the cells by centrifugation at 2,000–3,000 rpm for 5 min. Wash the cells with PBS/DPBS if necessary, count the cells, and transfer approximately 5 × 10⁶ cells to a fresh 1.5 ml tube.

- 2) Centrifuge at 13,000 rpm for 10–20 sec to harvest the cell pellet.

Note : After centrifugation, carefully remove the supernatant without disturbing the cell pellet.

- 3) Add 400 µl of PRO-PREP™ Solution and resuspend the cell pellet thoroughly.

Note : Approximately 400 µl of PRO-PREP™ Solution per 5 × 10⁶ cells is recommended. The volume may be adjusted depending on the cell type and pellet size. Mix carefully to minimize bubble formation.

- 4) Incubate on ice or at -20°C for 10–20 min.

Note : For more efficient cell lysis, the sample may be passed through a syringe before incubation. Bubbles generated during mixing generally disappear during incubation or centrifugation.

[Tissues]

- 1) Prepare approximately 10–20 mg of fresh tissue.

Note : Keep the tissue fresh and cold during sample preparation.

- 2) Add 600 µl of PRO-PREP™ Solution.

Note : Approximately 600 µl of PRO-PREP™ Solution per 10 mg of tissue is recommended. The volume may be adjusted depending on the tissue type and sample amount.

- 3) Homogenize the tissue thoroughly.

Note : Keep the sample cold during homogenization. Bubbles generated during homogenization generally disappear during incubation or centrifugation.

- 4) ncubate on ice or at -20°C for 10–20 min.

Note : For more efficient lysis, the homogenized sample may be passed through a syringe before incubation.

6.3. Protein Extraction

- 1) Centrifuge the lysate at 13,000 rpm (4°C) for 5 min.

- 2) Transfer the supernatant to a fresh 1.5 ml tube.

Note : Carefully collect the supernatant without disturbing the pellet.

- 3) Use the collected supernatant for protein quantification or downstream applications.

7. TROUBLESHOOTING GUIDE

Problem	Possible cause	Recommendation
Low protein yield	Insufficient cell number or incomplete lysis	Check the sample amount and PRO-PREP™ Solution volume. If necessary, extend the lysis time and mix periodically.
Protein degradation	Improper sample handling	Keep samples and PRO-PREP™ Solution cold during protein extraction and avoid prolonged exposure to room temperature.
Excessive bubbles	Pipetting or homogenization	Bubbles generally disappear during incubation or centrifugation and do not affect protein extraction.
PRO-PREP™ Solution is frozen	Storage temperature is too low	Check the freezer temperature. Thaw the solution before use and maintain the recommended storage temperature.
Crystals or precipitate in the solution	Precipitation during low-temperature storage	Allow the solution to dissolve briefly at room temperature, then place it on ice before use.

8. EXPERIMENTAL INFORMATION

8.1. Protein Extraction Yield

[Cultured Cell]

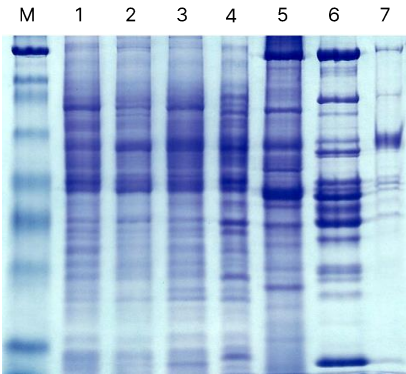
Cell Line	Cell Number	Protein
K562 (Human)	5 × 10 ⁶	2.04 mg
SNU 601 (Human)	5 × 10 ⁶	1.89 mg
YAC 1 (Mouse)	5 × 10 ⁶	2.10 mg
B16 (Mouse)	5 × 10 ⁶	1.90 mg

[Tissues]

Tissue	Amount	Protein
Spleen (Mouse)	10 mg	9.01 mg
Kidney (Mouse)	50 mg	9.91 mg
Lung (Mouse)	10 mg	9.67 mg
Liver (Mouse)	10 mg	6.59 mg

8.2. SDS PAGE Gel Analysis

Total proteins extracted from various cultured cells and tissues using PRO-PREP™ Protein Extraction Solution were analyzed by 15% SDS-PAGE and visualized by Coomassie Brilliant Blue staining.



Lane M, PRO-STAIN™ (I) Marker; Lane 1, K562; Lane 2, SNU 1; Lane 3, SNU 5; Lane 4, B16; Lane 5, Rat Heart; Lane 6, Cow Muscle; Lane 7, Cow Subcutaneous Fat.

9. RELATED PRODUCT

Product	Cat. No
SMART™ BCA Protein Assay Kit	21071
PRO-MEASURE™ Protein Measurement Solution	21011
Golden-STAIN™ Prestained Protein Ladder	24053
Miracle-Femto™ Western Detection System	16035
Miracle-Star™ Western Blot Detection System	16025
NomelRT™ Western Blot Stripping Buffer	21112