PIPPR® Total Mammalian Protein Extraction Kit

Cat. No.: C40101

Item	PIPPR® Total Mammalian Protein Extraction Kit
ID No.	**4800100 + 4800200 + 4800300**
Kit Content	Mammalian Cell Lysis Buffer + Benzonase® Nuclease 25U/µl, 2000U +100x Protease Inhibitor Solution
Size	100 extractions

The PIPPR® Total Mammalian Protein Extraction Kit provides gentle but efficient detergent-based lysis of mammalian cells to deliver a soluble protein fraction suitable for any downstream application. Cell pellets are incubated with Mammalian Cell Lysis Buffer supplemented with Benzonase® — an enzyme that digests nucleic acids — and a protease inhibitor, which protects the released proteins from enzymatic degradation. No specialized equipment is required. Cells are simply incubated with lysis buffer and centrifuged to separate cell debris. The supernatant contains the total protein fraction, which can be further purified or directly analyzed, for example by SDS-PAGE or MS.

Table 1. Protein Yields and Protein Solubilization Using the PIPPR® Total Mammalian Protein Extraction Kit

Cell Line	Protein Yield (ug)*	Solubilisation (%)*
HeLa	1700	97
Cos-7	900	93
Jurkat	780	96
NIH	940	97
CHO	800	98

* Average of 3 preparations, each of 5 x 10^6 cells.

Key Features

- Gentle but effective cell lysis for high yields of active proteins
- Simple procedure, no specialized equipment required
- Highly reproducible procedure, consistent results time after time
- Optimised for the Proteomic Expression analysis Platform , PIPPR® based on SWATH LC-MS

Recommended Storage and Stability

All kit components should be stored at 2–8°C. For longer storage, Benzonase® Nuclease should be stored at -20°C

Quality Control

In accordance with COBO Technologies Quality Management System, each lot of PIPPR® Total Mammalian Protein Extraction Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The PIPPR® Total Mammalian Protein Extraction Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of COBO Technologies products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.cobotechnologies.com where you can find, view, and print the MSDS for each COBO Technologies kit and kit component.

Protocol: Isolation of Total Proteins from Mammalian Cells

The volumes given in this protocol are suitable for processing of 5–10 x 10^6 mammalian cells. When processing larger or smaller cultures, adjust the volume of buffer used accordingly.

Equipment and reagents be supplied by the user

- Ice-cold PBS
- Cell scraper
- 15 ml conical tube
- Microcentrifuge tubes
- Microcentrifuge
- Rotary shaker

Important notes before starting

It is possible that the Protease Inhibitor does not thaw at room temperature. If this is the case, heat the protease inhibitor solution to 37°C with agitation and cool to room temperature.

When processing small volumes, it may be necessary to dilute Benzonase® Nuclease using Mammalian Lysis Buffer.

Procedure

Cell collection

1. Aspirate cell-culture medium from culture plate.
2. Wash cells twice with 5 ml ice-cold PBS.
3. Add 10 ml ice-cold PBS.
4. Remove cells from culture plate by gentle scraping with cell-scraper and transfer cells to a pre-chilled 15 ml conical tube.
5. Centrifuge cell suspension for 5 min at 450 x *g* in a centrifuge pre-cooled to 4°C. Discard supernatant. Keep cell pellet on ice.

Cell lysis

6. Add 1 U Benzonase Nuclease and 10 µl of Protease Inhibitor (100x) to 1 ml Mammalian Lysis Buffer.
7. Resuspend 5–10 x 10^6 cells in the Mammalian Lysis Buffer prepared in step 6 and incubate on a rotary shaker for 5 minutes at 4°C.
8. Centrifuge the suspension for 10 minutes at 14,000 x *g* in a microcentrifuge tube pre-cooled to 4°C.
9. Transfer the supernatant into a new microcentrifuge tube. The supernatant contains the total protein fraction. For some applications, such as 2-D gel analysis, the protein fraction may need to be concentrated. This can be achieved by acetone precipitation (see below).

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications.

1. Add four volumes of ice-cold acetone to the protein fraction and incubate for 15 min on ice.
2. Centrifuge for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Discard the supernatant and air dry the pellet. *Do not overdry the pellet as this may make it difficult to resuspend*.
3. Depending on the application, resuspend the pellet in the required sample buffer. *For 2D-PAGE, an extra desalting step may be required*.
4. Resuspend the pellet from step 2 in 100 µl 8M urea.
5. Desalt the sample using a gel filtration device (e.g., Bio-Spin® 6, Bio- Rad cat. no. 732-6227).
6. Repeat steps 1 to 3.

Troubleshooting

Comments and Suggestions Inconsistent results in protein quantification assays

The extraction buffer contains components that may interfere with protein quantification assays. A solution to this would be to perform a precipitation step (e.g., using acetone, that see above) to remove interfering substances.

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