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ProteoExtract® Transmembrane Protein Extraction Kit

About the Kit 2
Overview 2
Components 2
Storage 2
Equipment and materials required but not supplied 3

ProteoExtract® Transmembrane Protein Extraction Protocol 3
Extraction of membrane proteins from adherent cultured cells 3
Extraction of membrane proteins from suspension cells or frozen cell pellets 4
Extraction of membrane proteins from tissue 6

Frequently Asked Questions 8

Appendix 8
Example extractions 8
Examples of total protein yields using TM-PEK 9

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About the Kit

ProteoExtract® Transmembrane Protein Extraction Kit	1 kit	71772-3

Overview

The ProteoExtract® Transmembrane Protein Extraction Kit (TM-PEK) uses a novel, detergent-free chemistry for extraction of peripherally-associated and integral membrane proteins, such as G-Protein Coupled Receptors (GPCRs), from mammalian cells and tissues. The membrane protein fraction is directly compatible with enzyme assays, native and denaturing gel electrophoresis, Western blotting, immunoprecipitation, and (following in-gel tryptic digestion) mass spectrometry.

The TM-PEK method comprises a two-step protocol for the enrichment of transmembrane (TM) proteins. In the first step, cells or homogenized tissues are permeablized using Extraction Buffer 1 and their soluble (cytoplasmic) fraction separated from the insoluble (membrane) fraction by centrifugation. In the second step, membrane proteins are extracted from the lipid bilayer using one of two novel extraction buffers. These buffers, Extraction Buffer 2A and Extraction Buffer 2B, are prepared by diluting TM-PEK Reagent A or TM-PEK Reagent B into Extraction Buffer 2. The optimal extraction reagent must be determined empirically, as it will depend on characteristics of the protein(s) of interest and intended downstream applications. Extraction Buffer 2A is a very mild extraction agent, allowing for recovery of fragile protein complexes. Extraction Buffer 2B, by comparison, is a highly efficient extraction agent and facilitates recovery of difficult-to-extract transmembrane proteins, including those with multiple transmembrane segments.

If using the TM-PEK kit for the first time, prepare duplicate samples. Extract the first set of replicates with TM-PEK Reagent A, diluted 2-fold with Extraction Buffer 2. Extract the second set of replicates with TM-PEK Reagent B, diluted 2-fold with Extraction Buffer 2. Extraction conditions can be optimized further by varying the dilution range of each reagent into Extraction Buffer, from undiluted to a 10-fold dilution.

Unlike alternative membrane protein extraction methods, the TM-PEK kit does not require sonication, rigorous vortexing, time-consuming ultracentrifugation, or incubation at elevated temperatures. The absence of such harsh treatments minimizes potential damage or changes to the target protein.

Each TM-PEK kit provides sufficient reagents to process a total of 40 samples (20 samples using Extraction Buffer 2A at the recommended dilution, and 20 samples using Extraction Buffer 2B at the recommended dilution). Each sample may be comprised of 1–5 x 10^7 cultured cells or 25–50 mg of tissue

Components

- 40 ml Extraction Buffer 1
- 50 ml Extraction Buffer 2
- 2.0 ml TM-PEK Reagent A
- 2.0 ml TM-PEK Reagent B
- 0.4 ml Protease Inhibitor Cocktail Set III

Storage

Store all components at 4°C for up to 6 months. For prolonged storage, dispense the components in working aliquots and store at –20°C. Before performing extractions, thaw all kit components at room temperature and mix completely. Avoid repeated freezing and thawing.

Note that the protease inhibitors are provided in DMSO and must be kept at room temperature during the extraction procedure to prevent freezing.

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Equipment and materials required but not supplied

- Rocking platform or elliptical mixer (required at room temperature and 4°C; the device can be transferred from 4°C to room temperature during the course of the experiment)
- Homogenizer (e.g., Dounce or Potter-Elvehjem) or mortar and pestle (for tissue samples)
- Refrigerated centrifuge with rotor accommodating a 15 ml and/or a 50 ml tube
- Refrigerated centrifuge with rotor accommodating a 2 ml tube and generating 16,000 × g
- Phosphate Buffered Saline (PBS) (137 mM NaCl, 10 mM Na2HPO4, 2.7 mM KCl, 1.8 mM KH2PO4, pH 7.4)

ProteoExtract® Transmembrane Protein Extraction Protocol

Table 1. Buffer volumes required for membrane protein extraction from cultured cells or tissue.

Source Material	Cultured cells*	Fresh or frozen tissue		
Amount	1.0–5.0 x 10^7	25–50 mg	100–200 mg	200–1000 mg
Extraction Buffer 1 (ml)	1.0	1.0	1.0	2.0
Extraction Buffer 2A *or* 2B (ml)	0.2	0.2	0.5	1.0
Protease Inhibitor (µl)	5	5	20	40

***Adherent, suspension, or frozen pellet**

Extraction of membrane proteins from adherent cultured cells

Considerations Before You Begin

The following protocol is optimized for extraction of membrane proteins from adherent cells grown in one T-75 culture flask. Cells should be of high viability (> 90%) and 70–90% confluent (1.0–2.0 x 10^7 cells). Different cell types yield considerably different amounts of protein in the membrane protein fraction (see Table 2 on p 9). If low yield is anticipated, the total number of cells can be increased to 5.0 x 10^7 without increasing reagent volumes (Table 1). For membrane protein extraction from larger cell numbers, it is recommended to perform replicate extractions from aliquots of 1.0–5.0 x 10^7 cells. Alternatively, buffer volumes may be scaled up appropriately.

The kit is supplied with two transmembrane solubilization agents, TM-PEK Reagents A and B (see Overview on p 2). Depending on the unique characteristics of the target membrane protein, Extraction Buffer 2A or Extraction Buffer 2B may prove to be more efficient. As a starting point, it is recommended to test both membrane extraction buffers. If performing trial extractions using both reagents, carry out Steps 2–9 in parallel on each of two sets of sample replicates.

Extraction Buffer 2A is a mild extraction reagent which can facilitate recovery of protein complexes. Thus, protein yields obtained using Extraction Buffer 2A may be lower than those obtained using Extraction Buffer 2B (see Table 2 on p 9). Extracts can be concentrated with an Amicon® Ultra-2 mL or Amicon® Ultra-0.5 mL Ultrafiltration Centrifugal Device.

Large volumes of TM-PEK 2B reagent can interfere with protein migration in SDS-PAGE. If high resolution is desired, Extraction Buffer 2B samples for SDS-PAGE may be prepared in 2X SDS sample buffer. Alternatively, employ a buffer exchange step.

For most transmembrane proteins, a 2-fold dilution of TM-PEK Reagent A or B in Extraction Buffer 2 results in efficient extraction. For some transmembrane proteins, extraction efficiency may be improved by using TM-PEK Reagent A or B at a different concentration, from undiluted to a 10-fold dilution in Extraction Buffer 2.

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Protocol

1. Prepare Extraction Buffer(s) 2A and/or 2B by diluting the appropriate TM-PEK Reagent 2-fold with Extraction Buffer 2. For membrane protein extraction from 1.0–5.0 x 10^7 cells, 0.2 ml of Extraction Buffer 2A or 0.2 ml of Extraction Buffer 2B is required.
   - To prepare 0.2 ml of Extraction Buffer 2A, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent A.
   - To prepare 0.2 ml of Extraction Buffer 2B, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent B.

   Notes: It is recommended to test both Extraction Buffer 2A and Extraction Buffer 2B to determine which is optimal for your protein of interest.

   Samples prepared with Extraction Buffer 2A may require concentration, depending on downstream application.

   High levels of Reagent B can interfere with protein migration on SDS-PAGE. Buffer exchange or dilution in sample buffer may be required.

   A 2-fold dilution of TM-PEK Reagent A or B in Extraction Buffer 2 results in an efficient extraction of most proteins, but reagent dilutions may be optimized (from undiluted to a 10-fold dilution).
2. Discard medium from the culture flask.
3. Wash cells two times with PBS at 4°C.
4. Add 3 ml PBS to the culture vessel. Using a cell scraper or a rubber policeman, free cells from the culture vessel. Transfer cells to a 15 ml conical tube.
5. Centrifuge cells at 1000 × g for 5 min at 4°C. (Alternatively, collect cells by centrifuging at 500 × g for 10 min at 4°C.)
6. Resuspend cells in 1 ml Extraction Buffer 1 + 5 µl Protease Inhibitor Cocktail Set III.
7. Incubate 10 min at 4°C with gentle agitation to avoid formation of cell clumps.
8. Centrifuge at 1000 × g for 5 min at 4°C.
9. Carefully remove supernatant. Store on ice. This is referred to as ‘cytosolic (soluble)’ protein fraction.
10. Resuspend pellet in 0.2 ml Extraction Buffer 2A + 5 µl of Protease Inhibitor Cocktail Set III or 0.2 ml Extraction Buffer 2B + 5 µl of Protease Inhibitor Cocktail Set III.
11. Incubate for 45 min at room temperature with gentle agitation.

    Note: The length and temperature of the incubation at Step 11 can be varied to improve extraction efficiency or to preserve target protein activity. Increasing the incubation time (up to 120 min) can increase the protein recovery, but may result in decreased target protein activity. Conversely, incubation at low temperature (4°C) may better preserve activity, but may lower the extraction efficiency.
12. Centrifuge at 16,000 × g for 15 min at 4°C.
13. Transfer the supernatant, which is enriched in integral membrane proteins, to a fresh tube.

    Note: Store cytosolic and membrane fractions on ice if they will be analyzed on the same day. For long-term storage, dispense into aliquots and store at –20°C.
14. Determine total protein concentration of the cytosolic and membrane protein fractions with the BCA assay.

    Note: For some cell types, total protein concentration of the membrane protein fraction (Step 13 above) will be >1.0 mg/ml. A two- to four-fold dilution in sterile, deionized water may be required to bring the concentration of these samples within the linear region of the BCA standard curve.

Extraction of membrane proteins from suspension cells or frozen cell pellets

Considerations Before You Begin

The following protocol is optimized for extraction of membrane proteins from 1.0–2.0 x 10^7 cells cultured in suspension. Cells should be of high viability (>90%). Different cell types yield considerably different amounts of protein in the membrane protein fraction (see Table 2 on p 9). If low yield is anticipated, increase the total number of cells to 5.0 x 10^7 without increasing reagent volumes (see Table 1 on p 3.). For extracting membrane proteins from larger cell numbers, perform replicate extractions from aliquots of 1.0–5.0 x 10^7 cells. Alternatively, scale up buffer volumes.

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The TM-PEK kit is also compatible with frozen cell pellets (1.0–5.0 x 10^7 cells per extraction). Cells should be washed a minimum of two times with an appropriate buffer (e.g., PBS) prior to freezing in liquid nitrogen. If using frozen cell pellets, begin at Step 7 below.

The kit is supplied with two transmembrane solubilization agents, TM-PEK Reagents A and B (see Overview on p 2.). Depending on the unique characteristics of the target membrane protein, Extraction Buffer 2A or Extraction Buffer 2B may prove to be more efficient. As a starting point, it is recommended to test both membrane extraction buffers. If performing trial extractions using both reagents, carry out Steps 2–9 in parallel on each of two sets of sample replicates.

Extraction Buffer 2A is a mild extraction reagent which can facilitate recovery of protein complexes. Thus, protein yields obtained using Extraction Buffer 2A may be lower than those obtained using Extraction Buffer 2B (see Table 2 on p 9). Extracts can be concentrated with an Amicon® Ultra-2 mL or Amicon® Ultra-0.5 mL Ultrafiltration Centrifugal Device.

Large volumes of TM-PEK 2B reagent can interfere with protein migration in SDS-PAGE. If high resolution is desired, Extraction Buffer 2B samples for SDS-PAGE may be prepared in 2X SDS sample buffer. Alternatively, employ a buffer exchange step.

For most transmembrane proteins, a 2-fold dilution of TM-PEK Reagent A or B in Extraction Buffer 2 results in efficient extraction. For some transmembrane proteins, extraction efficiency may be improved by using TM-PEK Reagent A or B at a different concentration, from undiluted to a 10-fold dilution in Extraction Buffer 2.

Protocol

1. Prepare Extraction Buffer(s) 2A and/or 2B by diluting the appropriate TM-PEK Reagent 2-fold with Extraction Buffer 2. For membrane protein extraction from 1.0–5.0 x 107 cells, 0.2 ml of Extraction Buffer 2A or 0.2 ml of Extraction Buffer 2B is required.

   - To prepare 0.2 ml of Extraction Buffer 2A, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent A.
   - To prepare 0.2 ml of Extraction Buffer 2B, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent B.

   Notes: It is recommended to test both Extraction Buffer 2A and Extraction Buffer 2B to determine which is optimal for your protein of interest.

   Samples prepared with Extraction Buffer 2A may require concentration, depending on downstream application.

   High levels of Reagent B can interfere with protein migration on SDS-PAGE. Buffer exchange or dilution in sample buffer may be required.

   A 2-fold dilution of TM-PEK Reagent A or B in Extraction Buffer 2 results in an efficient extraction of most proteins, but reagent dilutions may be optimized (from undiluted to a 10-fold dilution).
2. Transfer 1.0–5.0 x 10^7 cells to a centrifuge tube.
3. Centrifuge cells at 1000 × g for 5 min at 4°C. (Alternatively, collect cells by centrifuging at 500 × g for 10 min at 4°C.)
4. Discard supernatant and gently resuspend cells in 5 ml PBS (4°C).
5. Centrifuge cells at 1000 × g for 5 min at 4°C.
6. Repeat Steps 4 and 5 two times, for a total of three washes.
7. Centrifuge cells at 1000 × g for 5 min at 4°C.

   Note: At this point, cells may be frozen in liquid nitrogen and stored at –70°C.
8. Resuspend cells in 1 ml Extraction Buffer 1 + 5 µl Protease Inhibitor Cocktail Set III.
9. Incubate 10 min at 4°C with gentle agitation to avoid formation of cell clumps.
10. Centrifuge at 1000 × g for 5 min at 4°C.
11. Carefully remove supernatant. Store on ice. This is referred to as the ‘cytosolic (soluble)’ protein fraction.
12. Resuspend pellet in 0.2 ml Extraction Buffer 2A + 5 µl Protease Inhibitor Cocktail Set III or 0.2 ml Extraction Buffer 2B + 5 µl Protease Inhibitor Cocktail Set III.
13. Incubate for 45 min at room temperature with gentle agitation.

    Note: The length and temperature of the incubation at Step 13 can be varied to improve extraction efficiency or to preserve target protein activity. Increasing the incubation time (up to 120 min) can increase the protein recovery,

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but may result in decreased target protein activity. Conversely, incubation at low temperature (4°C) may better preserve activity, but may lower the extraction efficiency.

14. Centrifuge at 16,000 × g for 15 min at 4°C.
15. Transfer supernatant, which is enriched in integral membrane proteins, to a fresh tube.

    Note: Store cytosolic and membrane fractions on ice if they will be analyzed on the same day. For long-term storage, dispense into aliquots and store at –20°C.
16. Determine the total protein concentration of the cytosolic and membrane protein fractions with the BCA assay.

    Note: For some cell types, total protein concentration of the membrane protein fraction (Step 15 above) will be >1.0 mg/ml. A two- to four-fold dilution in sterile, deionized water may be required to bring the concentration of these samples within the linear region of the BCA standard curve.

Extraction of membrane proteins from tissue

Considerations Before You Begin

The following protocol is optimized for extracting membrane proteins from 25–50 mg of fresh or frozen tissue. If tissue is not limiting, start with 100–1000 mg of tissue to offset sample loss during homogenization. When using > 50 mg of tissue, increase volumes of the extraction buffers (see Table 1 on p 3 for buffer volume guidelines). As an example, ~2 mg total protein can be extracted from 35 mg bovine liver (see Table 3 on p 9 for representative yields from other tissue sources and types). Yields from various tissue types can vary considerably, however. Certain transmembrane proteins are expressed at very low levels in various tissues, and thus may be below the lower limit of detection by immunological methods. In this circumstance, analysis by mass spectrometry may prove beneficial.

Protocol

1. Prepare membrane Extraction Buffer(s) 2A and/or 2B by diluting the appropriate TM-PEK Reagent 2-fold with Extraction Buffer 2. For membrane protein extraction from 25–50 mg of tissue, 0.2 ml of Extraction Buffer 2A or 0.2 ml of Extraction Buffer 2B is required.
    - To prepare 0.2 ml of Extraction Buffer 2A, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent A.
    - To prepare 0.2 ml of Extraction Buffer 2B, mix 0.1 ml Extraction Buffer 2 and 0.1 ml TM-PEK Reagent B.

    Note: It is recommended to test both Extraction Buffer 2A and Extraction Buffer 2B to determine which is optimal for your protein of interest.

    Samples prepared with Extraction Buffer 2A may require concentration, depending on downstream application.

    High levels of Reagent B can interfere with protein migration on SDS-PAGE. Buffer exchange or dilution in sample buffer may be required.

    A 2-fold dilution of TM-PEK Reagent A or B in Extraction Buffer 2 results in an efficient extraction of most proteins, but reagent dilutions may be optimized (from undiluted to a 10-fold dilution).
2. Ensure that all buffers are thawed and well mixed. Keep Extraction Buffers 1, 2A, and/or 2B on ice during the extraction procedure. Keep the Protease Inhibitor Cocktail Set III at room temperature to prevent DMSO from freezing.
3. Following dissection of the tissue of interest, quickly remove unwanted materials (e.g., connective tissue, fat, blood vessels, etc). To slow proteolysis, keep tissue at 4°C while refining the dissection.
4. Quickly slice the tissue into ~2 mm^3 pieces. Add tissue slices to a tube containing 2 ml ice-cold PBS.
5. Gently flick tube to dislodge blood cells and other loosely attached material.
6. Collect tissue pieces by centrifuging at 100 × g for 2 min at 4°C. Remove and discard the supernatant.
7. Repeat Steps 5 and 6 for a total of two washes. After the second wash, ensure that all PBS has been removed completely.

    Notes: At this point, the tissue can be frozen on liquid nitrogen and stored at –70°C.

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During tissue extraction, it is important to work quickly, but carefully. Keep the sample cool (<4°C) and store buffers on ice throughout the extraction procedure.

8. Transfer the tissue (fresh or frozen) to a pre-cooled homogenizer. Ideally, a glass Potter-Elvehjem or Dounce homogenizer is recommended.
9. Add 5 µl Protease Inhibitor Cocktail Set III to the wall of the homogenizer.
10. Add 2 ml ice-cold Extraction Buffer 1 to the homogenizer.
11. Carefully homogenize until tissue is completely homogenized and intact pieces are no longer visible. Use as few strokes as possible (e.g., ~10 strokes for 50 mg mouse liver). The number of strokes will depend on the type of tissue used. If desired, homogenization efficiency can be monitored by phase contrast microscopy. An efficient homogenization should generate small cell clumps rather that fragmentation of individual cells.

    Note: Some tissues (e.g., heart, muscle, brain) may be difficult to completely dissociate by mechanical homogenization. As an alternative, the ProteoExtract® Tissue Dissociation Buffer Kit (Cat. No. 539720) including collagenase may be used. Other tissue-specific protocols may be compatible with the TM-PEK kit.
12. Incubate 10 min at 4°C with gentle agitation.
13. Centrifuge at 1000 × g for 5 min at 4°C.
14. Carefully remove supernatant. Store on ice. This is referred to as the 'cytosolic (soluble)' protein fraction.
15. Add 5 ml ice-cold PBS. Gently resuspend pellet. Collect membranes by centrifuging at 1000 × g for 5 min at 4°C. Carefully remove supernatant.

    Note: When using > 200 mg tissue, perform an extra wash at this point to remove additional cytosolic proteins. Repeat Step 15 for a total of two washes.
16. Completely and carefully resuspend pellet in 0.2 ml Extraction Buffer 2A + 5 µl of Protease Inhibitor Cocktail Set III or 0.2 ml Extraction Buffer 2B + 5 µl Protease Inhibitor Cocktail Set III.
17. Incubate 15 min at 4°C with gentle agitation to avoid formation of cell clumps.
18. Centrifuge at 16,000 × g for 15 min at 4°C.
19. Transfer supernatant, which is enriched in integral membrane proteins, to a fresh tube.

    Note: Store cytosolic and membrane fractions on ice if they will be analyzed on the same day.
    For long-term storage, dispense into aliquots and store at –20°C.
20. Determine the total protein concentration of the cytosolic and membrane fractions with the BCA assay.

    Note: For some tissue types, total protein concentration of the membrane protein fraction (Step 19 above) will be >1.0 mg/ml. A two- to ten-fold dilution in sterile, deionized water may be required to bring the concentration of these samples within the linear region of the BCA standard curve.

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Frequently Asked Questions

Question	Answer
How do I determine the protein concentration of the membrane protein extract?	The components in the extraction buffers are directly compatible with common protein assays. It is recommended to use the BCA Protein Assay Kit (Cat. No. 71285-3). Note that the Extraction Buffer 2B fraction may require a 2-4-fold dilution to bring the total protein concentration within the linear range of the BCA assay.
How do I prepare the TM-PEK fraction for one-dimensional SDS-PAGE?	The TM-PEK fractions can be analyzed directly by one-dimensional SDS-PAGE. For samples extracted with Reagent A, add SDS-PAGE sample buffer to a 1X final concentration and load directly on to the gel. Samples extracted with Reagent B should be prepared using a 2X concentration of SDS-PAGE sample buffer.
How can I concentrate the TM-PEK extracted proteins?	It is possible to reduce the volume of Extraction Buffer 2A or 2B used, but this may decrease the total protein yield. It is recommended to use the ProteExtract® Protein Precipitation Kit (Cat. No. 539180) to concentrate samples for use in downstream applications not requiring native protein. For applications that do require native protein, it is recommended to use an ultrafiltration device such as an Amicon® Ultra-2 mL or Amicon® Ultra-0.5 mL Ultrafiltration Centrifugal Device.
How should the membrane fractions be treated prior to mass spectrometry analysis?	The membrane extracts should be applied to a one-dimensional or two-dimensional gel, spots or bands cut from the gel, and then digested with trypsin.

Appendix

Example extractions

In the examples below, the SDS extract (total cell lysate) serves as a positive control. The Extraction Buffer 2A recovers EGFR, which has a single transmembrane span, with efficiency comparable to TRITON® X-100. However, Extraction Buffer 2A recovers Frizzled-4 and CELSR-3, both of which contain seven transmembrane domains, with far greater efficiency than does TRITON X-100.

A: EGFR B: Frizzled-4 C: CELSR-3

Figure 1. Extraction of transmembrane proteins from MDA-MB-468 breast adenocarcinoma cultured cells. Transmembrane proteins were extracted from MDA-MB 468 cells using the TM-PEK kit. In the first step, two identical pools of 1 x 10^7 cells were treated with Extraction Buffer 1, which recovers proteins from the cytosolic fraction. The insoluble material was then treated with TM-PEK Extraction Buffer 2A or 0.5% TRITON® X-100. Lane 1 shows the expected size of the target proteins and is not a quantitative control. Lanes 2-4 each contain protein extract from 1 x 10^6 cells, which is one-tenth of the 1 x 10^7 cells originally harvested. Fractions were separated using a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. Membranes were blocked and incubated with primary antibody to EGFR (panel A), Frizzled-4 (panel B) or CELSR-3 (panel C). Blots were developed using an HRP-conjugated secondary antibody and a chemiluminescent substrate. Lane 1, 0.5% SDS (total cell lysate); Lane 2, cytoplasmic fraction; Lane 3, membrane fraction (TRITON X-100); Lane 4, membrane fraction (Extraction Buffer 2A). Arrows indicate size of the full-length version of each protein.

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Examples of total protein yields using TM-PEK

The values presented in Tables 2 and 3 are intended to serve as guides for estimating the required amount of starting material. For each cultured cell type, proteins were extracted according to the TM-PEK protocol from cell monolayers grown to 80% confluency in a T-75 flask. All tissues were disrupted mechanically using a Dounce homogenizer.

Table 2. Protein yields for cytosolic and membrane fractions recovered from cultured cells using the TM-PEK kit.

Cell Type	Total Protein (mg/10^7 cells)*		
	Extraction Buffer 1 *(cytosolic)*	**Extraction Buffer 2A** *(membrane)*	**Extraction Buffer 2B** *(membrane)*
MDA-MB-468 *(breast adenocarcinoma)*	0.62	0.15	0.78
MCF 7 *(breast adenocarcinoma)*	0.68	0.26	1.27
A-431 *(epidermoid carcinoma)*	0.55	0.12	0.75
CHO-K1 *(Chinese hamster ovary)*	0.52	0.22	0.94
NCI-H292 *(mucoepidermoid carcinoma)*	0.06	0.04	0.47
HEP-G2 *(hepatocellular carcinoma)*	0.97	0.13	0.76
Mia PaCa-2 *(pancreatic carcinoma)*	0.23	0.05	0.52
HCT 116 *(colon carcinoma)*	0.60	0.10	0.76

*Protein concentrations were determined using the BCA assay.

Table 3. Protein yields for cytosolic and membrane fractions recovered from mouse tissues using the TM-PEK kit.

Tissue Type	Total Protein (µg/mg tissue)*		
	Extraction Buffer 1 *(cytosolic)*	**Extraction Buffer 2A** *(membrane)*	**Extraction Buffer 2B** *(membrane)*
Liver§	55.6	1.7	11.4
Heart	28.2	2.9	17.3
Brain	18.4	0.9	5.8
Spleen§	31.6	8.2	8.8
Skeletal Muscle	13.6	2.1	6.3
Kidney	33.6	5.4	16.9

*Protein concentrations were determined using the BCA assay.

§Average of two

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