Total Protein Extraction Kit

Introduction
CytoMol's total protein extraction kit is an excellent tool for the initial purification and preparation of proteins from any tissues and cells. Total protein isolated by this kit is native and can be used for downstream applications including SDS-PAGE, Western blotting, gel mobility shift, protein assays and other procedures. The kit is easy to use: no scraping, no freeze-thaw cycles, and no sonication.

Features

- Easy protocol
- Isolate protein from large samples
- Rapid isolation without freeze and thaw and sonication
- Isolate protein either from tissues or from cultured cells
- Proteins isolated by this kit are intact and could be over 200 kd in mass (See Figure 1 on next page)

Description
The Total Protein Extraction Kit is composed of two buffers: TM and 50X PI. Before isolation of total proteins from tissues or cells, simply dilute the 50X PI buffer with TM buffer according to the instruction in the manual. The kit can be used for isolation of total proteins from 5 gram of tissue or 125 million cultured cells. **Minimal 0.1 g tissue or 2.5 million cells should be required.**

Application
The Total Protein Extraction Kit can be used for isolation of protein from tissues or cultured cells. The isolated protein can be used for Western Blot, DNA-protein interaction, enzymatic activity analysis, protein-protein interaction, and immunoprecipitation and tissue specific expression identification.

Components

Item	Component	Amount	Part No.
BufferTM	HEPES (pH7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium deoxycholate, NP-40, Sodium OrthoVanadate	13 ml	pke110111
50 x PI	A cocktail of protease inhibitors	260 µl	pke110112

Using Total Protein Extraction Kit
The following guidelines should be considered before using the kit to extract proteins.

- Aliquot 50X PI on ice and store them at -20ºC
- Always make fresh working solutions before the isolation of proteins
- Buffer TM is stored at 4ºC

1. Dilute 50X PI solution to 1X PI in TM buffer, and keep the solution on ice.
2. Weigh certain amount tissues and chop them in small pieces.
3. Keep the tissues on dry ice.
4. Add 1X PI to the tissue at **2.5 ml per gram tissue or per 25 million cells**, and put on ice for 5 minutes
5. Homogenize the tissue or cells for 20 seconds, and then put it on dry ice for 15 seconds.
6. Homogenize the tissue or cells for the 2nd 20 seconds. (The 3rd 20 seconds homogenization may be required if the tissue or cell is not well homogenized.)
7. Rotate the homogenized tissue or cells at 4˚C for 20 minutes.
8. Centrifuge at 18,000 g at 4˚C for 20 minutes.
9. Collect the supernatant.

10. Determine the concentration of the total proteins.

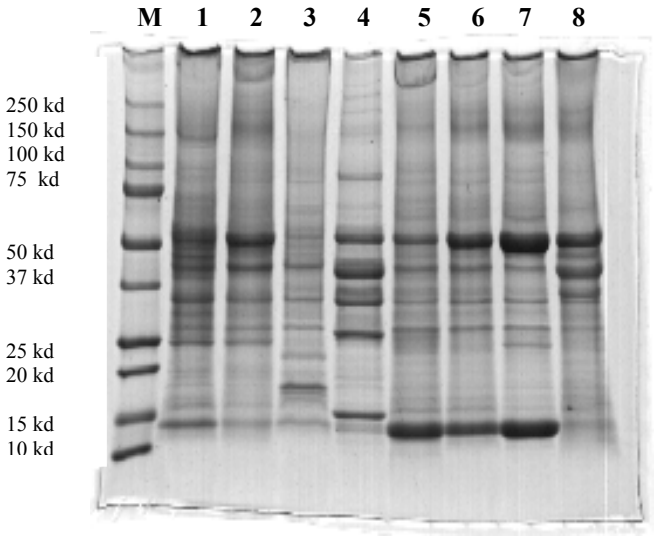

Figure 1. The image of 50 µg total proteins on 4-20% gradient SDA-PAGE gel. Total proteins were isolated from 8 different human tissues by Total Protein Extraction Kit. Lane 1, Liver; lane 2, Kidney; lane 3, Brain; lane 4, Skeletal Muscle; lane 5 Spleen; lane 6, Lung; lane 7, Placenta; and lane 8, Stomach.