




ProteoExtract™ Kits

Reproducible Protein Extraction

Advancing your life science discoveries™

www.calbiochem.com technical@calbiochem.com Or Contact Your Local Sales Office

CB0716-2003NP

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Choosing the right protein extraction procedure

One of the major challenges in proteomics is the separation of complex protein mixtures for quantitative analysis as well as for the detection of differential post-translational modifications or differential subcellular localization of proteins depending on environmental parameters.

Prerequisites for the success of such an analysis are standardized and reproducible operating procedures for sample preparation prior to one- and two-dimensional gel electrophoresis and/or liquid chromatography. The sample preparation method of choice has to achieve the reproducible solubilization of all types of proteins, the preservation of the protein pattern by preventing protein degradation, as well as an efficient removal of contaminating nucleic acids.

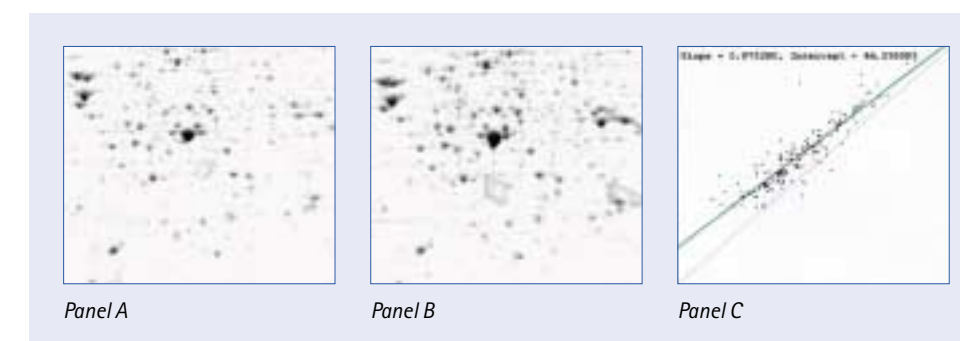
Due to the complexity of the proteome and the divergence of protein properties it is necessary to prepare standardized partial proteomes of a given organism in order to maximize the coverage of the proteome and to increase the chance of visualizing low-abundance proteins and making them accessible for subsequent analysis. Utilizing sequential proteome extraction schemes, differences in solubility and sub-cellular localization can be exploited.

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ProteoExtract™ Kits are manufactured by Merck KGaA, Darmstadt, Germany

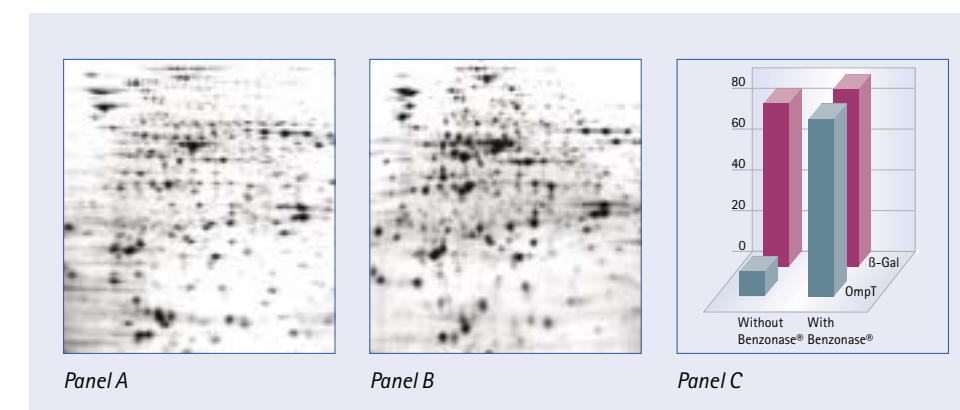
ProteoExtract™ Kits

The newly developed ProteoExtract™ Kits are designed for fast and reproducible extraction of proteins from a wide range of sources including bacteria, yeast, tissue culture cells, and mammalian tissues. Different kits have been tailored to suit different protein separation needs.

All kits contain proteomics grade ultra-pure chemicals & buffers to provide optimal resolution and reproducibility in 2D gel electrophoresis-protein patterns. Benzonase®, a proprietary non-specific nuclease, is included for efficient nucleic acid degradation in order to reduce sample viscosity and increase spot resolution. The Subcellular and the Partial Proteome Extraction Kits contain ready-to-use Protease Inhibitor Cocktail to preserve the entire protein profile.



Standardized operating procedures with high quality reagents lead to high reproducibility of proteome extraction. Panel A + B show two independent results from proteome extraction of E. coli. Panel C indicates that the two independent experiments are highly correlated ($R = 0.92$).

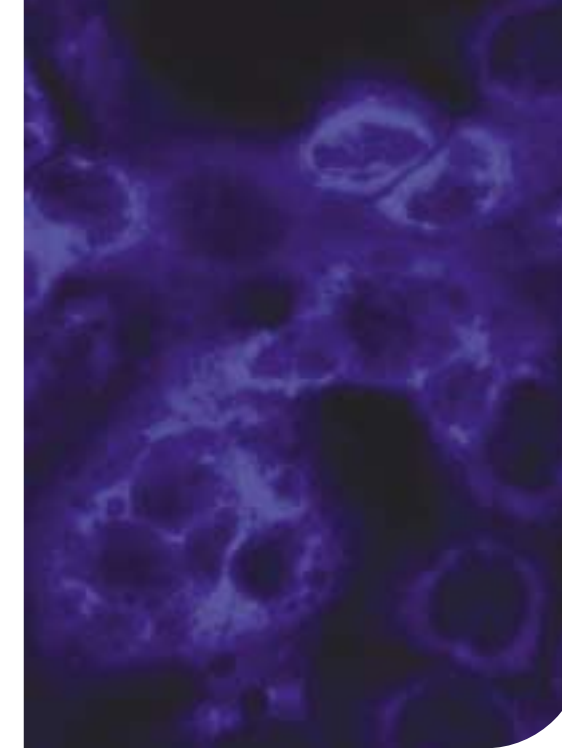


Efficient removal of contaminating nucleic acids by treatment with Benzonase® leads to better spot resolution in 2D electrophoresis and reduces the risk of precipitation of less soluble proteins.

Panel A + B show a result from a proteome extraction of E. coli without (A) or with (B) treatment of the extract with Benzonase®, a proprietary DNase/RNase from Merck KGaA. Nuclease treatment leads to non-viscous cell extracts and better spot resolution. Panel C indicates an improved yield of hydrophobic proteins (e.g. OmpT) after treatment of cell extracts with Benzonase®.

ProteoExtract™ Kits Features and Benefits

- Efficient & reproducible protein extractions
 - Optimized protocols for different biological samples
 - Saves time and eliminates laborious optimization work
- Optimal preparation for further downstream analysis
 - All-in-one solution for 20 samples per kit
 - Protease Inhibitor Cocktail improves results in 2D gel electrophoresis
- Better spot resolution by use of protease-free Benzonase®
- Ready-to-use, high quality protein extracts



ProteoExtract™ Subcellular Proteome Extraction Kit (S-PEK)



ProteoExtract™ Subcellular Proteome Extraction Kit

Is the first kit that provides fast and reproducible extraction of subcellular proteomes from mammalian tissue culture cells. The kit utilizes an innovative extraction buffer system* that allows monitoring of the subcellular localization of proteins e.g. signaling proteins. Because the proteomes are obtained in their native state Subcellular Kit extracted proteins are ideally suited for use in direct functional assays or protein arrays. Each kit contains the reagents for 20 extractions.

* Patent pending



ProteoExtract™ Partial Proteome Extraction Kits

Are designed for highly efficient sequential extraction of cellular proteins according to their solubility. The step-wise solubilization of different subsets of cellular proteins allows visualization of more protein spots in total and detection of low abundance protein.

Three Partial Proteome Extraction Kits are offered with special configuration and protocols for bacteria, yeast and mammalian tissue & tissue culture cells. Each kit contains all reagents needed to perform 20 sample preparations.



ProteoExtract™ Complete Proteome Extraction Kits

Are ideally suited to isolate total proteins for a broad overview of proteomes and fast screening. The procedures result in reproducible yields of total cellular proteins ready for direct analysis on 2DGE gels without further protein concentration.

Three distinct kit formats are available, providing optimized configuration and protocols for bacteria, yeast and mammalian tissue & tissue culture cells. Each kit contains the reagents for 20 reactions.

For fast & reproducible extraction of subcellular proteomes from adherent as well as suspension-grown mammalian cells that allows detection of proteins localized in different subcellular compartments. Purified proteins are obtained in the native state making the S-PEK suitable for many downstream applications such as 1DGE/2DGE, immunoblotting, and enzyme activity assays.

Features & Benefits

- Stepwise extraction resulting in four distinct subcellular proteomes from one sample
- Highly reproducible
- A simple, no ultracentrifugation steps
- Fast – needs just 2 hours with 45 minutes hands-on time
- Produces native, functional proteins

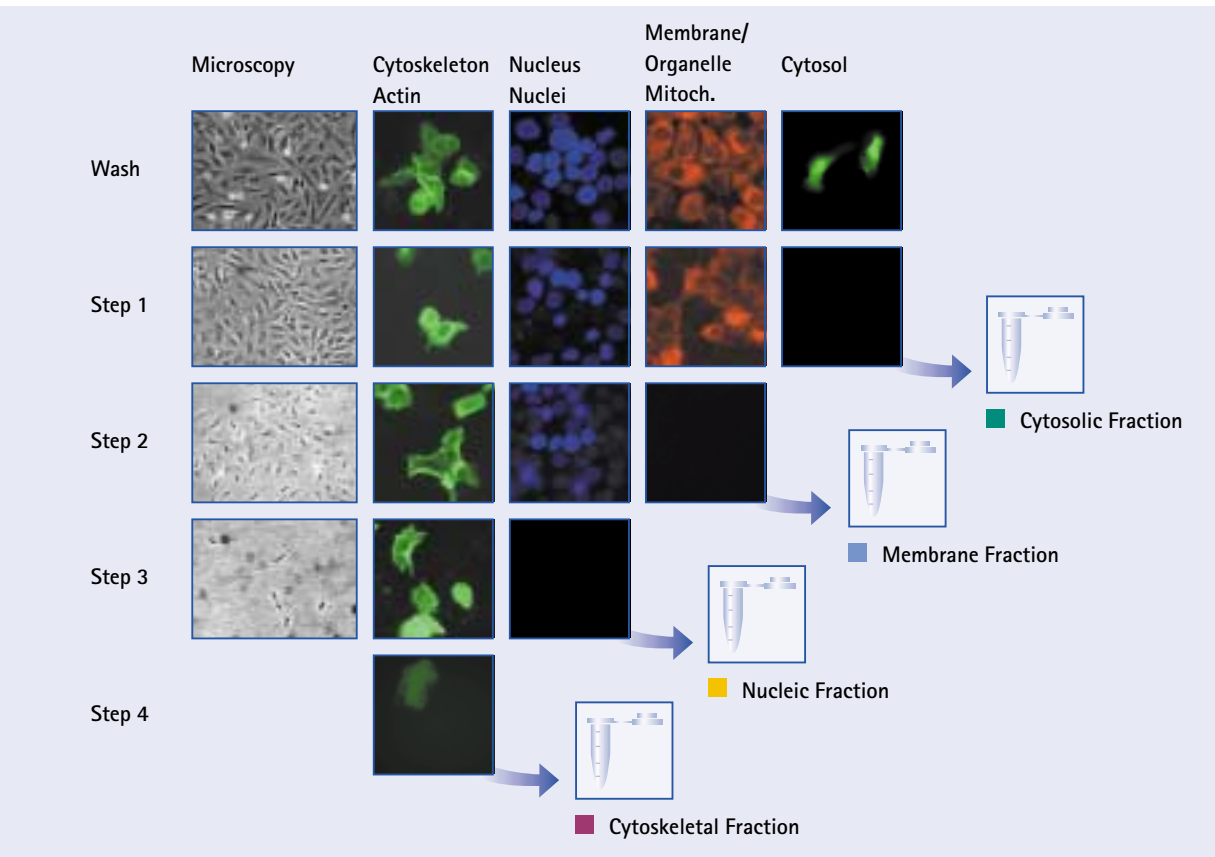
Principle

The S-PEK takes advantage of the different solubility of certain subcellular compartments in the four selected reagents. In case of adherent cells, the procedure is performed directly in the tissue culture dish without the need for cell removal. For suspension-grown cells, extraction starts with gentle sedimentation and washing of the cells. Cells or the parts of the cells remain attached to the plate during sequential extraction of subcellular compartments, until the appropriate extraction reagent is used. Thus, the early destruction of the cellular structure by enzymatic or mechanical detachment of cells from the tissue culture plate and any mixing of different subcellular compartments is prevented.

ProteoExtract™ Subcellular Proteome Extraction Kit (S-PEK)

Procedure

The stepwise extraction delivers 4 distinct protein fractions from one sample: Cytosolic fraction (F1), Membrane/organelle protein fraction (F2), Nucleic protein fraction (F3), Cytoskeletal fraction (F4).



Morphological changes of cells during subcellular extraction

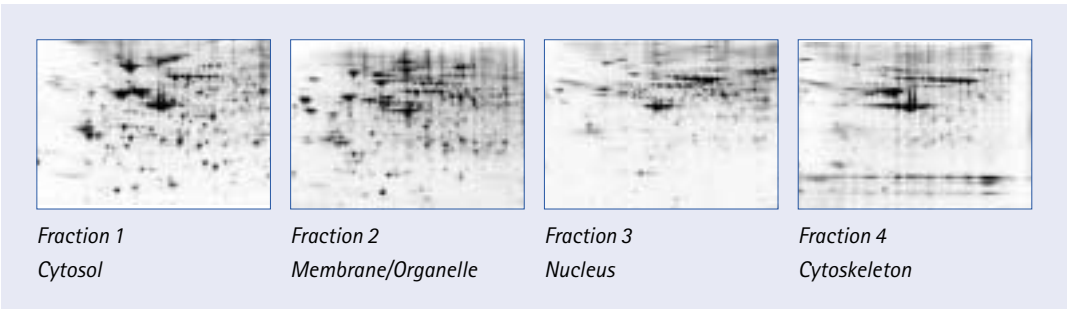
A431 cells were incubated with Phalloidin, DAPI and Mito Tracker™ in order to visualize actin filaments, nuclei and mitochondria, respectively. Stepwise extraction of cytosolic fraction (F1), organelle/membrane fraction (F2), nuclear fraction (F3) and cytoskeletal fraction (F4) monitored by fluorescence microscopy.

Applications

The innovative buffer system used in the S-PEK yields proteins directly suitable for various proteomics downstream applications. Because proteomes are obtained in the native state, proteins are particularly suited for sensitive applications such as enzyme activity assays or microarrays.

Protein profiling by 1D/2DGE

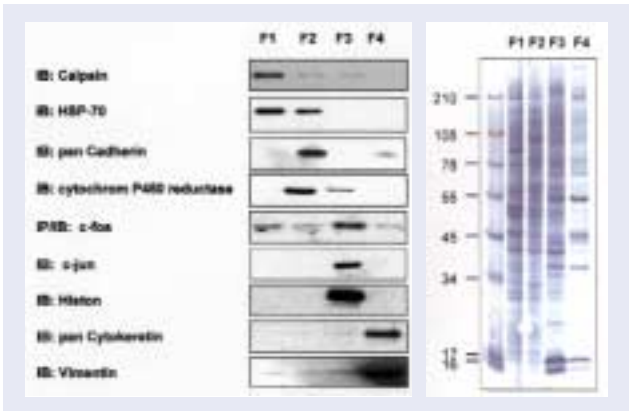
The S-PEK reveals clearly distinct protein profiles from different fractions.



The comparison of fractions 1–4 by two-dimensional SDS-PAGE indicates a large number of protein spots that are specific for the respective subcellular fraction.

Western Blotting

Stepwise extractions show clearly distinct protein patterns of different subcellular fractions.

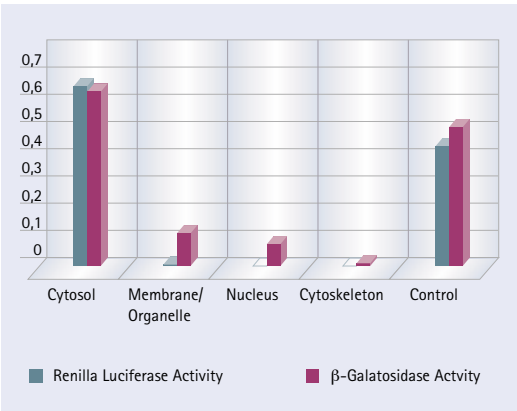


SDS-PAGE and Western blotting assign selected marker proteins to their expected subcellular fractions. More than 90 % of the marker proteins were detected in the corresponding subcellular fraction

Enzyme Activity Assays

Proteome fractions prepared with the S-PEK may be used for most enzyme assays including reporter gene assays, kinases, and immunoassays.

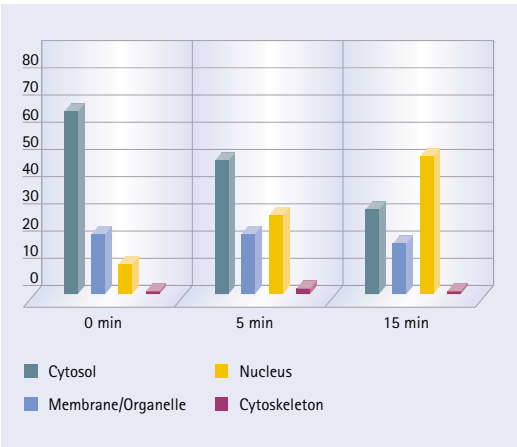
Cells were transfected with plasmids encoding either β -galactosidase or Renilla luciferase to be expressed in the cytoplasm. Subcellular extraction was performed and activities of the fractions 1 – 4 are displayed in arbitrary units using a dedicated commercially available extraction product as positive control.



Subcellular Redistribution Assays

Innovative extraction buffers in the S-PEK solubilize distinct subcellular compartments. The S-PEK provides a valuable tool for the evaluation and mechanistic studies of protein redistribution events, such as time and dose dependent relocation of signaling proteins upon stimulation of cells

A431 cells were stimulated with TNF α . Stepwise extraction of cytosolic fraction (F1), organelle/membrane fraction (F2), nuclear fraction (F3) and cytoskeletal fraction (F4) was performed using the subcellular proteome extraction kit. Cell fractions were separated by SDS-PAGE and the proteins blotted onto nitrocellulose membrane. The membrane was probed with anti-NF- κ B (p65, RelA). NF- κ B is localized in the cytoplasm of most cell types and transported to the nucleus upon stimulation.



Ordering Information

Product	Contents	Cat.No.
ProteoExtract™ Subcellular Proteome Extraction Kit	Reagents to process 20 samples, yielding 4 fractions each	539790

ProteoExtract™ Partial Proteome Extraction Kits (P-PEK)



Provides efficient & standardized extraction of cellular proteins into four distinct partial proteomes. Analysis of the partial proteome fractions enriched in subsets of cellular proteins allows for the detection of low abundance proteins and screening by means of 2D gel electrophoresis and western blotting.

Features & Benefits

- Specifically developed & tested protocols for different biological samples
- Protein fractions enriched in subsets of cellular proteins to visualize low abundance proteins
- Ready-to-use proteins for 2DGE
- Excellent spot resolution through Benzonase® addition
- Proteolysis inhibited to preserve protein profiles

Principle

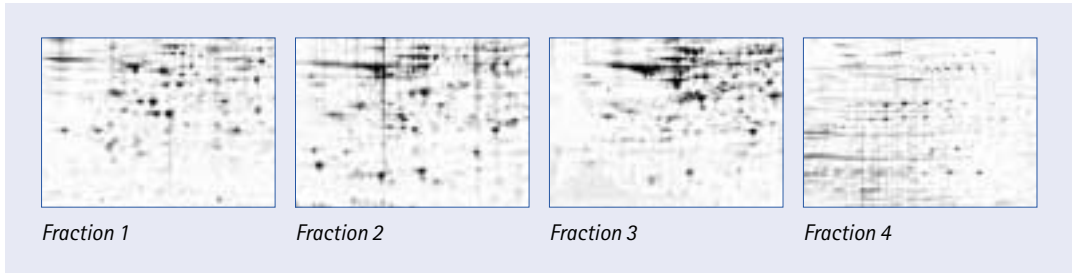
Partial Proteome Extraction Kits provide an all-in-one solution for a serial sample preparation of complex protein mixtures using buffers with increasing solubilization strength. P-PEK extraction reagents contain uniquely balanced combinations of chaotropes & detergents to solubilize and enrich a different subset of cellular proteins in each of four fractions. Analysis of the four partial proteome fractions increases the chance of visualizing low abundance proteins. The most soluble proteins are released by mechanical disruption in extraction reagent 1, while extraction reagent 2 releases proteins of intermediate solubility. The specially formulated reagent 3 provides efficient membrane protein extraction to give rise to fraction 3. Finally, SDS buffer solubilizes proteins still insoluble in reagent 3.

Effective removal of nucleic acids by Benzonase® eases sample handling and highly improves resolution on 2DGE gels. P-PEK protocols generally work without sonication step thus avoiding protein modifications by temperature effects. P-PEK extracted proteins are ready for immediate use in 2DGE applications.

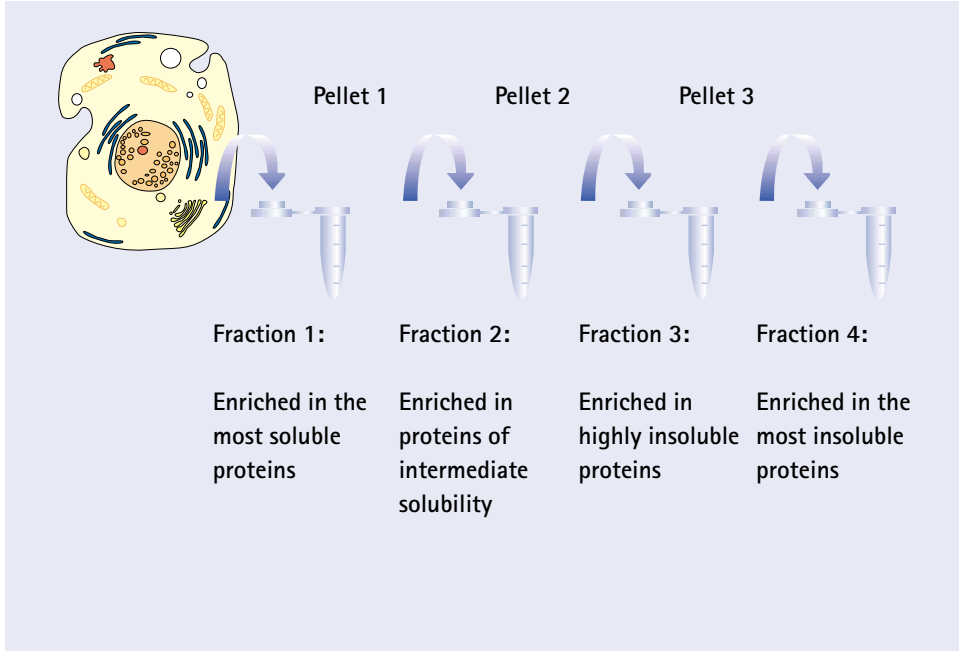
Special kit configurations and protocols have been developed for bacteria, yeast & mammalian samples eliminating time-consuming optimization steps.

Applications

- Protein extraction for gel separation techniques as 1D-/2DGE electrophoresis, Western blotting etc.
- Proteome prefractionation:
The sequential extraction of proteins according to their solubility results in distinct protein profiles for all four fractions.



Rat brain tissue was disrupted and homogenised. The homogenised tissue was subsequently extracted with the P-PEK method for partial mammalian proteome extraction. The comparison of fractions 1-4 by two-dimensional SDS-PAGE indicates a large number of protein spots being specific for the respective extraction fraction.



Procedure

Flow chart of partial proteome extraction from various kinds of biological materials. Biological samples are sequentially extracted with four buffers with increasing solubilization strength. The proteome of the organism of interest is partitioned into four partial proteomes enriched in proteins of increasing hydrophobicity

Ordering Information

Product	Contents	Cat.No.
ProteoExtract™ Partial Bacterial Proteome Extraction Kit	Reagents to process 20 bacterial samples, yielding 4 fractions each	539780
ProteoExtract™ Partial Yeast Proteome Extraction Kit	Reagents to process 20 yeast samples, yielding 3 fractions each	539785
ProteoExtract™ Partial Mammalian Proteome Extraction Kit	Reagents to process 20 mammalian samples, yielding 4 fractions each	539789

ProteoExtract™ Complete Proteome Extraction Kit (C-PEK)

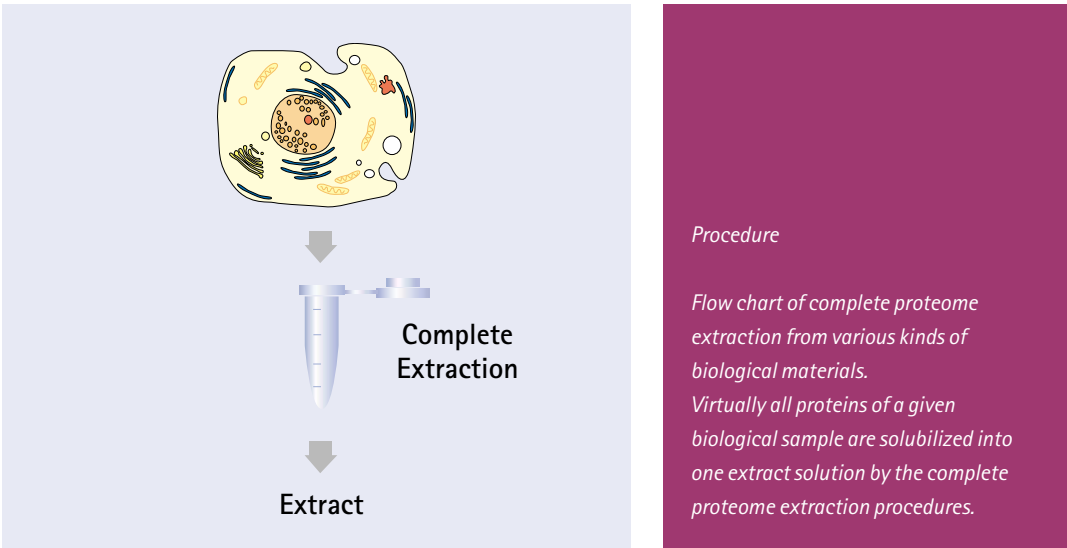
Designed for fast & easy extraction of total proteins from bacteria, yeast and mammalian cells & tissue, without the need for sonification or precipitation. C-PEK extracted proteins are ideally suited for fast screening by means of 2D overview gels or for free flow electrophoresis (FFE). Whenever specific detection tools such as antibodies are available, complete proteome extractions are ideally suited to visualize low-abundance proteins without additional enrichment schemes.

Features & Benefits

- Improved solubilization of cellular proteins
- Excellent quality of total proteins
- Specifically developed & tested protocols for different biological samples
- Ready-to-use proteins for 2DGE – no concentration needed
- Excellent spot resolution due to Benzonase® addition

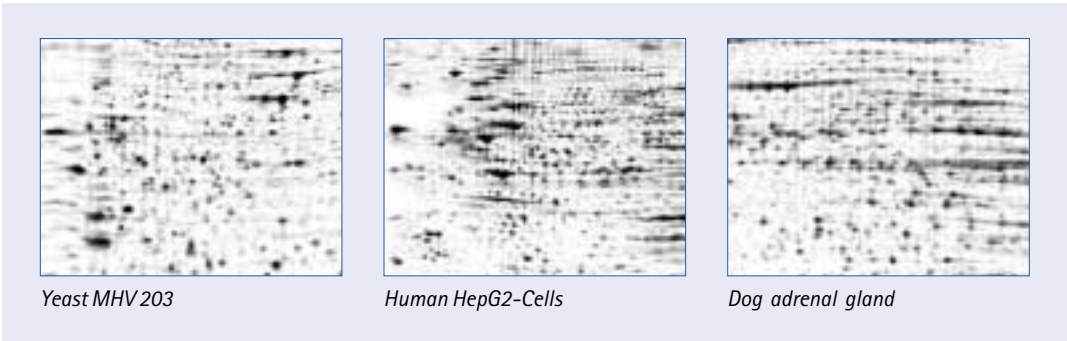
Principle

C-PEK provides a straightforward two-step isolation of complete proteomes in a single microcentrifuge tube. C-PEK uses optimized extraction reagents to provide improved solubilization of proteins resulting in an increased total number of spots on 2DGE gels. The procedure includes proprietary Benzonase®, a non-specific nuclease leading to clear, non-viscous protein solutions and improving resolution on 2DGE gels. Using the C-PEK procedure protein concentration is not necessary – extracted proteins are ready for immediate use in standard downstream proteomics applications. Special kits and protocols are available for bacteria, yeast & mammalian samples.



Applications

Extraction from cells and tissues with C-PEK results in a total protein sample that is ready for two-dimensional separation and ideally suited for overview or zoom gels.



Examples from different biological samples show high resolution in the 2 D gel electrophoresis.

Ordering Information

Product	Contents	Cat.No.
ProteoExtract™ Complete Bacterial Proteome Extraction Kit	Reagents to process 20 bacterial samples	539770
ProteoExtract™ Complete Yeast Proteome Extraction Kit	Reagents to process 20 yeast samples	539775
ProteoExtract™ Complete Mammalian Proteome Extraction Kit	Reagents to process 20 mammalian samples	539779