# SAMPLE PREPARATION

## ProteoPrep™ Membrane Extraction Kit

*For total extraction of membrane proteins*

This kit features new and innovative detergents, and uses specially formulated reagents and an optimized protocol to generate one fraction containing membrane proteins that is uniquely ready for two-dimensional (2D) electrophoresis. The special reducing and alkylating reagents produce samples that exhibit improved focusing and decreased streaking in 2D gels.

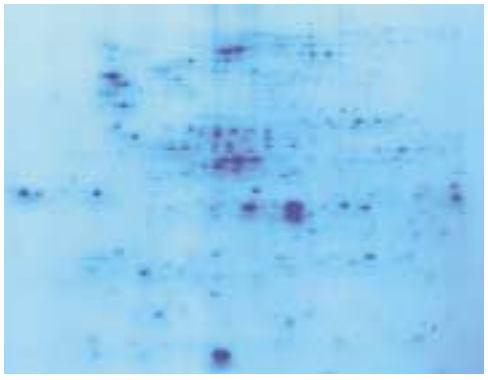
This kit provides reagents sufficient to process a minimum of six samples.

### Features & Benefits

- **Innovative detergent preparations** – *Improved solubility allows for higher protein loads and greater visibility of low abundance proteins in 2D gels*
- **Two pre-mixed solubilization solutions** – *Removes interfering non-membrane proteins prior to extraction, resulting in uncluttered 2D arrays*
- **Pre-measured reducing & alkylating reagents** – *Easy-to-use reagents provide improved IEF resolution*

| Components |
|---|
| Soluble Cytoplasmic and Loosely-Bound Membrane Protein Extraction Reagent |
| Cellular and Organelle Membrane Solubilizing Reagent |
| Tributylphosphine Stock Solution |
| Alkylating Reagent, Iodoacetamide |

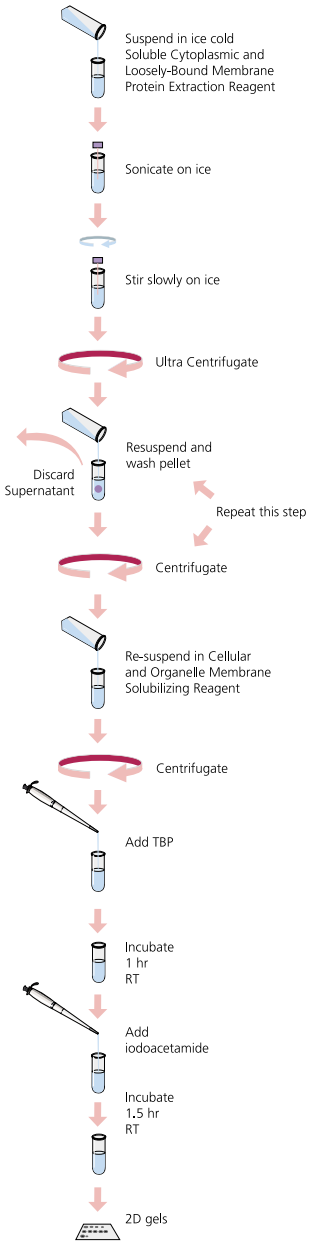
This kit is appropriate for use with various model organism sample sources used in proteomics research. Below is an illustration of the superior results produced from *E. coli* extractions. Higher protein loading capacities and improved solubility, especially for difficult membrane bound proteins, provide excellent visualization of low abundance/low copy proteins.



***Sample/Gel conditions***

*Source, E. coli, 1st dimension, IEF 4-7;*
*2nd dimension, 12% SDS-PAGE*

*Molloy, MP, et. al., Proteomic Analysis of the Escherichia Coli Outer Membrane., Eur. J. Biochem.,* ***267,*** *2871-2881(2000).*




| Product Code | Description | Size |
|---|---|---|
| PROT-MEM | ProteoPrep Membrane Extraction Kit | 1 kit |

Technology developed in partnership with Proteome Systems™

# SAMPLE PREPARATION



## ProteoPrep™ Universal Extraction Kit

*For the sequential isolation of separate soluble cytoplasmic and membrane protein fractions*

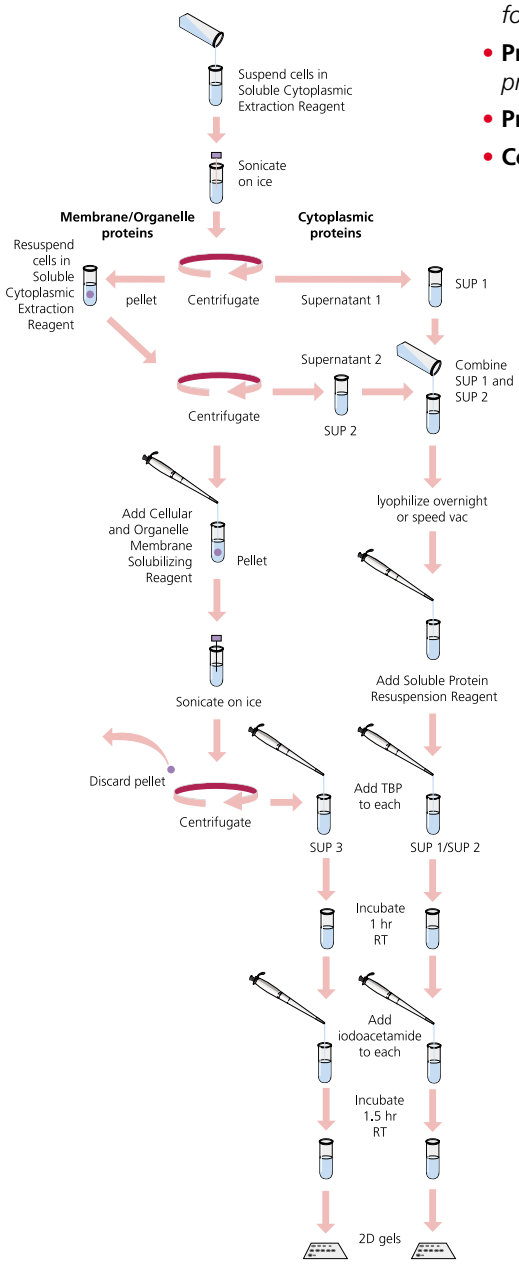
This kit features new and innovative detergents, and uses specially formulated reagents and an optimized protocol designed to generate two prepared subcellular fractions that are uniquely ready for two-dimensional (2D) electrophoresis.

- Fraction 1: Soluble/Cytoplasmic Proteins
- Fraction 2: Membrane Proteins

The special reducing and alkylating reagents produce samples that exhibit improved focusing and decreased streaking in 2D gels. This kit provides reagents sufficient to process a minimum of ten samples, yielding two fractions each.

### Features & Benefits

- **Innovative detergent preparations** – *Highly improved solubility allows for higher protein loads and greater visibility of low abundance proteins on 2D gels*
- **Two pre-mixed solubilization solutions** – *Generates two distinct populations for easy 2D analysis*
- **Pre-measured reducing and alkylating reagents** – *Easy-to-use reagents provide improved IEF resolution*
- **Pre-weighed dry blends** – *Stable and easy to reconstitute*
- **Conveniently Packaged** – *No waste. Use only the amount needed.*




| Components |
|---|
| Soluble Cytoplasmic Extraction Reagent |
| Soluble Protein Resuspension Reagent |
| Cellular and Organelle Membrane Solubilizing Reagent |
| Tributylphosphine Stock Solution |
| Alkylating Reagent, Iodoacetamide |

This kit is appropriate for use with various model organism sample sources used in proteomics research. Below is an illustration of the superior results produced from *E. coli* extractions. Improved solubility allows for higher protein loading capacities, resulting in improved visualization of low abundance proteins in 2D gels.

*Fraction 1*

*Fraction 2*

***Sample/Gel conditions***

*Source, E. coli, 1st dimension, IEF 4-7; 2nd dimension, 12% SDS-PAGE*

| Product Code | Description | Size |
|---|---|---|
| PROT-TWO | ProteoPrep Universal Extraction Kit | 1 kit |

Technology developed in partnership with Proteome Systems™

# SAMPLE PREPARATION

## ProteoPrep™ Sample Extraction Kit

*For testing or optimizing extraction conditions to produce total protein extracts*

This kit provides four extraction reagents of increasing solubilizing power (reagents 1→4), each of which can generate total protein extracts from cellular samples. Along with conventional reagents, the kit also includes the newest generation of detergent reagents. This allows comparison of the protein extractions obtained with each of the four reagents and optimization to meet your individual needs. The reducing and alkylating reagents produce protein samples that exhibit improved focusing and decreased streaking in 2D gels. Enough of each component is provided to process a minimum of ten samples by each extraction reagent.

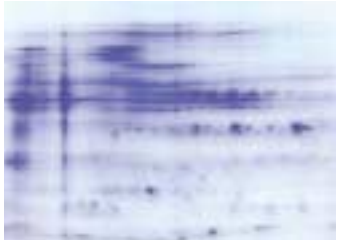
For researchers who have optimized an extraction protocol using one chaotropic extraction reagent, each reagent is available as an individual product as well.

### Features & Benefits

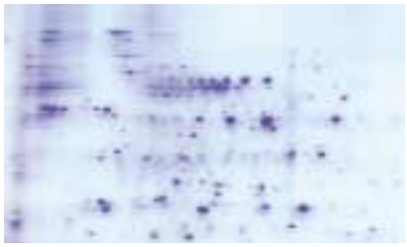
- **Four pre-mixed solubilization reagents** – *Enables rapid solublization*
- **Pre-measured reducing and alkylating reagents** – *Easy-to-use reagents provide improved IEF resolution*
- **Innovative detergent preparations** – *Highly improved solubility allows higher protein loads and greater visibility of low abundance proteins in 2D gels*

| Components |
|---|
| Chaotropic Membrane Extraction Reagent 1 |
| Chaotropic Membrane Extraction Reagent 2 |
| Chaotropic Membrane Extraction Reagent 3 |
| Cellular and Organelle Solubilizing Reagent 4 |
| Tributylphosphine Stock Solution |
| Alkylating Reagent, Iodoacetamide |

*Traditional Reagent*



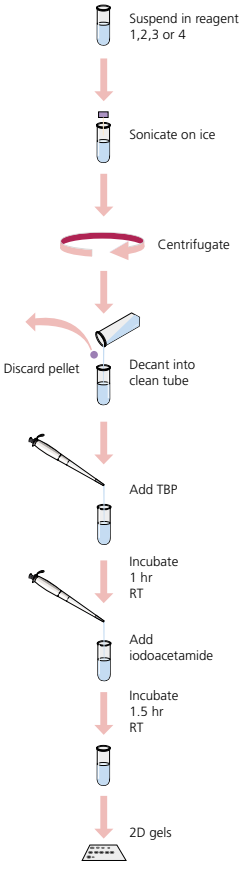
*ProteoPrep Reagent*



***Sample/Gel conditions***

*Source, E. coli, 1 mg protein load; 1st dimension, IEF 4-7; 2nd dimension, 12% SDS-PAGE*

*Herbert, B., Advances in protein solubilization for two-dimensional electrophoresis. Electrophoresis,* ***20,*** *660-663, (1999).*




*Observe the impact of each preparation by using equivalent process methods*

| Product Code | Description | Size |
|---|---|---|
| PROT-TOT | ProteoPrep Sample Extraction Kit | 1 kit |
| C 0481 | Chaotropic Membrane Extraction Reagent 1 | 4 btl |
| C 0606 | Chaotropic Membrane Extraction Reagent 2 | 4 btl |
| C 0731 | Chaotropic Membrane Extraction Reagent 3 | 4 btl |
| C 0356 | Cellular and Organelle Solubilizing Reagent 4 | 4 btl |
| T 7567 | Tributylphosphine Stock Solution | 10 vials |
| A 3221 | Alkylating Reagent, Iodoacetamide | 10 vials |

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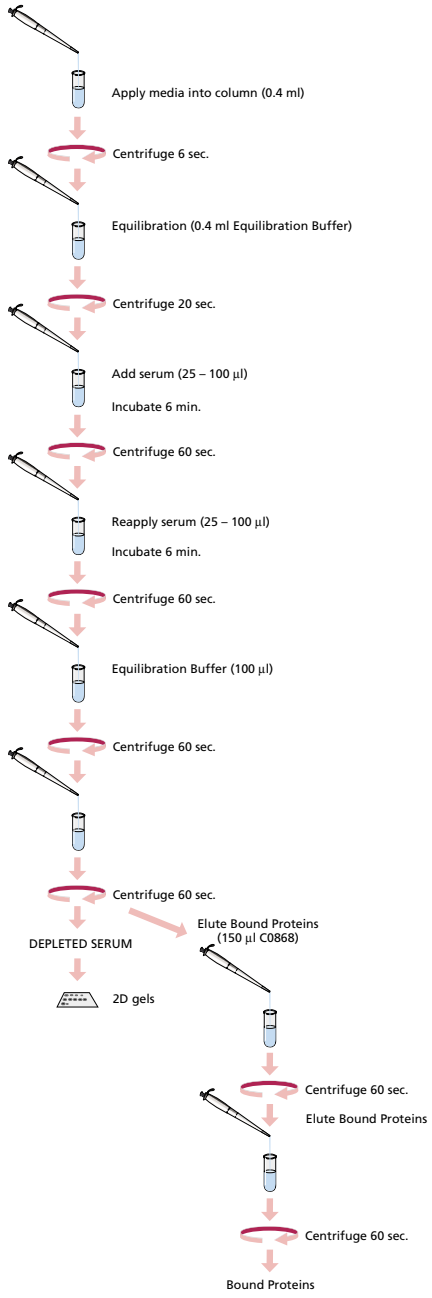
# SAMPLE PREPARATION






## ProteoPrep™ Blue Albumin Depletion Kit

Albumin and IgG are two major protein components of serum representing ~80% of the total serum protein. Removal from serum allows visualization of co-migrating proteins on a one dimensional electrophoresis or two dimensional electrophoresis gel and higher sample load (4 to 5-fold) for improved visualization of lower copy number proteins. ProteoPrep Blue Albumin Depletion Kit specifically removes albumin and IgG from 25 samples of human serum (25 µl to 100 µl) in preparation for two-dimensional electrophoresis. The ProteoPrep Blue Albumin Depletion Medium is a mixture of two media: 1) a proprietary blue matrix and 2) Protein G agarose. Typical depletions are 95% for albumin and 80% for IgG from 75 µl of human serum. The medium exhibits low non-specific binding because it does not contain Cibacron® Blue, notorious for high non-specific binding of non-albumin proteins.

The kit reagents are urea-based buffers instead of salt-based buffers meaning the albumin-depleted serum samples can be applied in two dimensional electrophoresis without protein precipitation. Focusing of proteins on immobilized pH gradient (IPG) strips for two dimensional electrophoresis of proteins and mass spec analysis of in-gel digested spots is negatively impacted (e.g. poor spot resolution) by the presence of salts or high concentrations of buffers. Many human serum albumin depletion products available today have salts in the equilibration and wash buffers typically requiring precipitation to remove the salts prior to two dimensional electrophoresis which is time consuming and risks loss of important proteins. Experiments show that urea can replace salts allowing for albumin binding to a proprietary dye-based resin and inhibiting binding of non-albumin proteins.

### Features & Benefits

- The ProteoPrep Blue media binds and removes greater than 85% of the albumin and greater than 75% of the IgG from 75 µl of serum allowing visualization of lower abundance proteins
- Equilibration buffer, a Tris-buffered urea solution, is two dimensional electrophoresis compatible and does not require sample precipitation
- Compatible with sample sizes less than 25 µl without undesirable non-specific binding

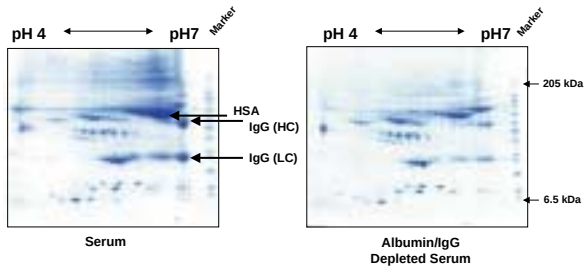



***Figure 1.*** *The two dimensional electrophoresis gels show the benefits for albumin and IgG depletion of serum. A 75 µl sample of human serum was depleted of albumin and IgG using the ProteoPrep Blue Albumin Depletion Kit. Two-dimensional electrophoresis was carried out on a 5 µl serum sample and the depleted serum using pH 4-7 IPG strips. The percent depletion of albumin and IgG was determined by ELISA to be 96% and 81% respectively.*

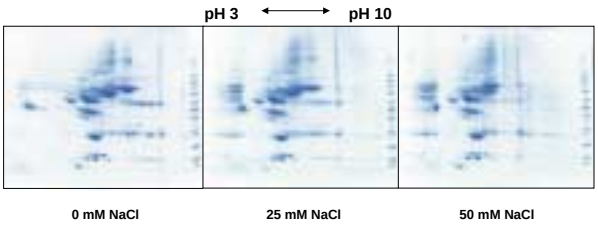



***Figure 2.*** *This figure demonstrates the negative impact that the presence of salts have on the resolution of proteins on a two dimensional electrophoresis gel.*

| Product Code | Description | Size |
|---|---|---|
| PROT-BA | ProteoPrep Blue Albumin Depletion Kit | 1 kit |

# SAMPLE PREPARATION

## Individual Detergents

| | Detergent | Product Code | Application |
|---|---|---|---|
| Nonionic | DDM (n-Dodecyl-β-D-maltoside) | D 4641 | Nonionic detergent for selective extraction[1, 2, 3] |
| | Octyl-β-D-glucopyranoside | O 8001 | Used for selective extraction; substitutes for SDS after 2D so proteins can be analyzed by MS[4, 5]; significantly increases the resolution of plant polypeptides in 2D gels[6]; preferred for myelin membrane proteins.[7] |
| | Octyl- β-D-Thioglucopyranoside | O 6004 | Dialyzable detergent, used for solublizing membrane proteins[8] and for dissociation of protein complexes.[9] |
| | TWEEN® 20, Sigma Ultra (low metal content) | P 7949 | Useful for removal of peripheral membrane proteins[10, 11] |
| | Saponin | S 4521 | Useful for permeabilizing or lysing cells,[12, 13] also as an adjuvant in vaccines[14] |
| Anionic | Sodium cholate hydrate | C 1254 | Useful for the extraction of membrane proteins[15, 16] |
| | Sodium dodecyl sulfate (SDS) | L 3771 | Useful for SDS-PAGE, must be at low concentration or removed prior to IEF[17] |
| | Sodium deoxycholate | D 6750 | Useful for the extraction of membrane receptors[18] and other plasma membrane proteins[19] and for nuclei isolation.[20] |
| Cationic | Hexadecyltrimethylammonium bromide (CTAB) | H 6269 | Useful for precipitating DNA[21, 22] and as a surfactant in drug/vaccine delivery systems[23] |
| | Trimethyl tetradecyl ammonium bromide (TTAB) | T 4762 | Useful as a surfactant in capillary electrophoresis[24, 25] |
| Zwitterionic | Aminosulfobetaine-14 (ASB-14) | A 1346 | Useful for the solubilization of proteins for analysis by 2D Electrophoresis[26, 27] |
| | C7BzO | C 0856 | Useful for the solubilization of proteins for analysis by 2D Electrophoresis[28, 29] |
| | CHAPS | C 9426 | Surfactant of choice for many IEF applications[30], solubilizes native membrane proteins[31, 32] and organelles[33] |
| | 3-(Decyldimethylammonio) propanesulfonate inner salt (SB3-10) | D 4266 | Useful for protein solubilization[34, 35] |
| | 3-(N,N-Dimethyloctadecylammonio) propane sulfonate (SB3-18) | O 8004 | Useful for extacting proteins for chromatographic, mass spec, and electrophoretic analysis[36, 37] |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# SAMPLE PREPARATION

## ProteoPrep™ Protein Precipitation Kit

The ProteoPrep™ Protein Precipitation Kit contains separate trichloroacetic acid (TCA) and sodium deoxycholate (DOC) reagents for the precipitation of proteins from aqueous samples. TCA is a classic reagent for precipitating proteins; however, it is not effective in quantitatively precipitating proteins below the 30 µg level. The use of TCA in combination with DOC allows for precipitation of as little as 3 µg of protein.

The kit provides reagents sufficient to precipitate fifty 0.1 ml protein samples.

### Features & Benefits

- **Fast –** *Precipitation in about 1 hour*
- **Convenient –** *All 3 reagents are provided as ready-to-use solutions*
- **Flexible –** *Resuspend pellet in the buffer of your choice to the protein concentration desired*
- **Compatible –** *Choice of resuspension buffer offers compatibility with a number of downstream processes such as SDS-PAGE, protein assay, IEF, tryptic digest etc.*
- **Scalable –** *Procedures allow for precipitation to as little as 3 µg of protein*

| Components |
|---|
| Trichloroacetic Acid, 100% (w/v) Solution |
| Deoxycholate, 0.2% (w/v) Solution |
| Wash Solution, 25% (v/v) Acetone Solution |

| Product Code | Description | Size |
|---|---|---|
| PROT-PR | ProteoPrep Protein Precipitation Kit | 1 kit |

ProteoPrep™ Reduction and Alkylation Kit, see page 97.