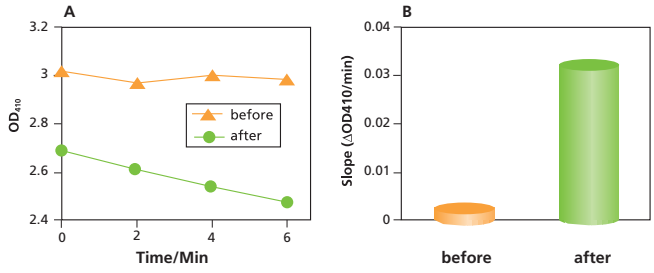
Protein Extraction for Proteomics



Ferricyanide photoreduction – a measure of intact chloroplasts
This assay is based upon the inability of the ferricyanide to cross the chloroplast envelope and to react with the electron transport system in the thylakoid membranes. Ferricyanide reduction, as indicated by the decrease in the absorbancy at 410 nm, occurs only when ruptured chloroplasts are present in the preparation.
The degree of integrity of the chloroplast preparation is assessed by comparing the rate of ferricyanide reduction upon illumination before and after osmotic shock of the chloroplasts.
Analysis of the results, presented here, indicates that 88% of the chloroplasts in the spinach chloroplast preparation are intact.
Chloroplasts (equivalent to 25 µg/ml chlorophyll) prepared using CP-ISO were illuminated in the presence of 1.5 mM ferricyanide. The reduction of ferricyanide was measured spectrophotometrically (410 nm).
Graph A demonstrates the change in absorbancy of the two samples (before and after osmotic shock) during 6 minutes.
Graph B shows bars representing the slopes of the lines in Graph A.

ProteoPrep™ Membrane Extraction Kit

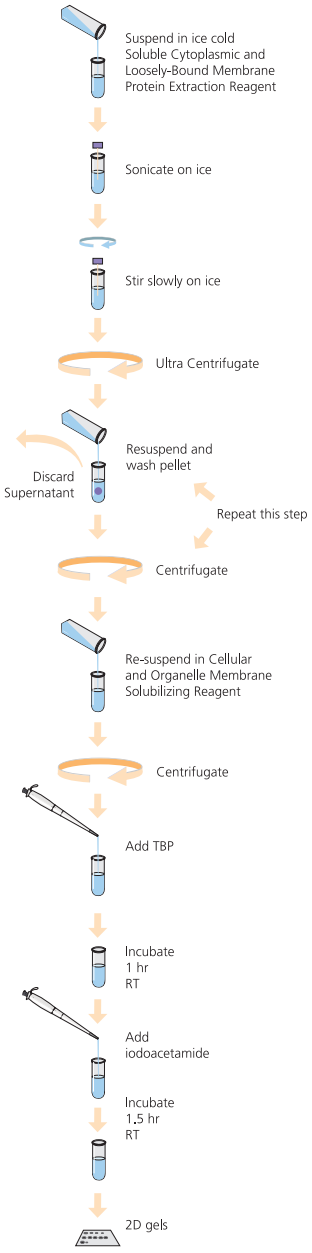
PROT-MEM 1 kit
2-8°C
NEW

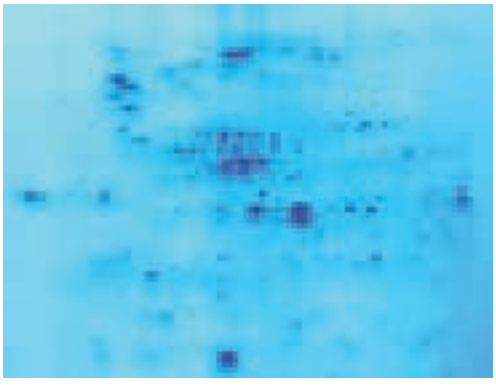
Designed to prepare a highly enriched membrane protein solution from many types of cells. The final protein solution is then suitable for 2D gel electrophoresis. The reagents are conveniently packaged and utilize a powerful new detergent for higher loading and high resolution of proteins in 2D gels. This kit also includes reagents for the reduction and alkylation of disulfide bonds. The ProteoPrep Membrane Extraction Kit was designed through a collaboration of **Proteome Systems** and Sigma research scientists.

References
1. Molloy, M.P., *Electrophoresis* **19**, 837 (1998)
2. Herbert, B.R., *Electrophoresis* **19**, 845 (1998)
R: 25-36/37/38-40-42/43-51/53-63 S: 26-36/37/39-45-61

Name	Units/Kit	Amt/Unit	Total/Kit
Soluble Cytoplasmic and Loosely-Bound Membrane Protein Extraction Reagent	3	125 ml*	375 ml*
Cellular and Organelle Membrane Solubilizing Reagent	1	23 ml*	23 ml*
Tributylphosphine Stock Solution	5	0.5 ml*	2.5 ml
Alkylating Reagent, Iodoacetamide	5	56 mg	280 mg

*Represents reconstituted volume





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

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

Protein Extraction for Proteomics

ProteoPrep™ Total Extraction Sample Kit

PROT-TOT 1 kit
2-8°C
NEW

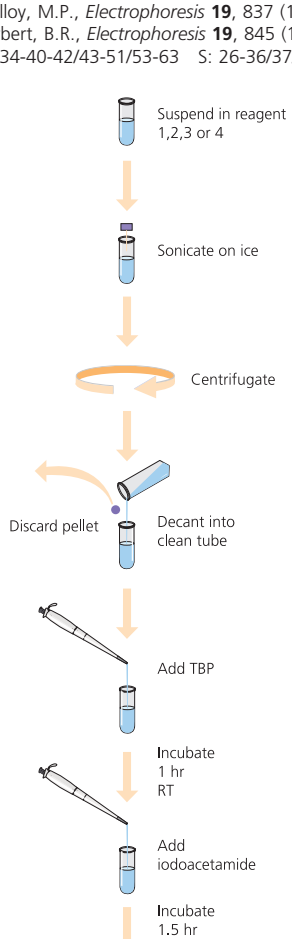
This kit provides four extraction reagents of increasing solubilizing power. Each can generate total protein extracts from cellular samples. Along with conventional reagents, the kit also includes the newest generation of detergent reagents. This allows comparison of the protein extractions obtained with each of the four reagents and optimization to meet your individual needs. The special reducing and alkylating reagents produce protein samples that exhibit improved focusing and decreased streaking in 2D gels. The ProteoPrep Total Extraction Kit was designed through a collaboration of **Proteome Systems** and Sigma research scientists.

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

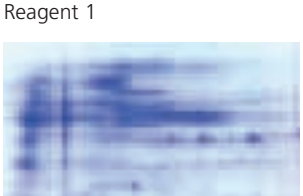
References

1. Molloy, M.P., *Electrophoresis* **19**, 837 (1998)
2. Herbert, B.R., *Electrophoresis* **19**, 845 (1998)

R: 25-34-40-42/43-51/53-63 S: 26-36/37/39-45-61



Reagent 1



Reagent 3

Reagent 2

Reagent 4

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).

ProteoPrep™ Universal Extraction Kit

PROT-TWO 1 kit
2-8°C
◆
NEW

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. The ProteoPrep Universal Extraction Kit was designed through a collaboration of **Proteome Systems** and Sigma research scientists.

References

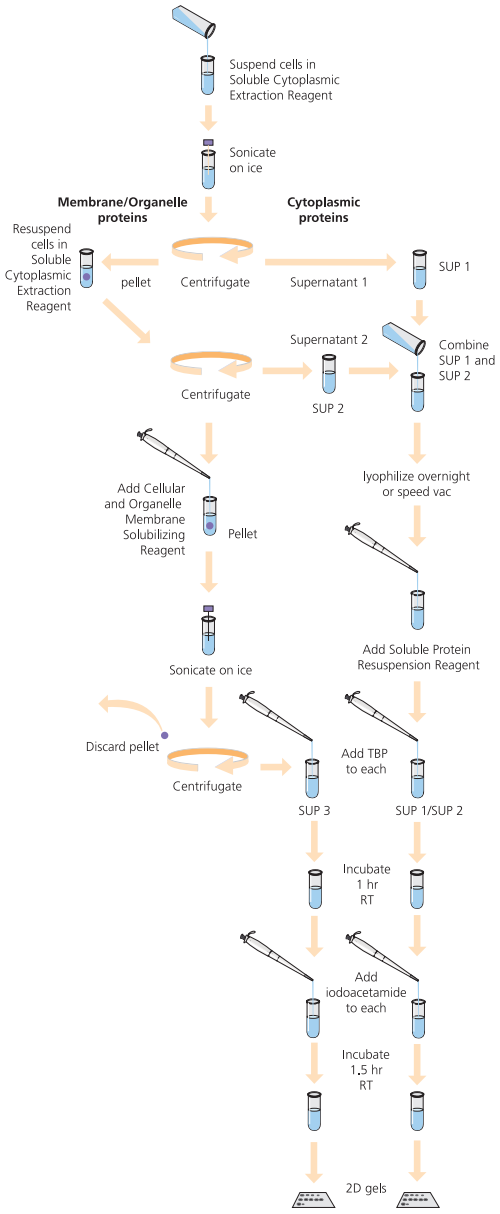
1. Molloy, M.P., *Electrophoresis* **19**, 837 (1998)
2. Herbert, B.R., *Electrophoresis* **19**, 845 (1998)

R: 25-34-40-42/43-51/53-63 S: 26-36/37/39-45-61

Name	Units/Kit	Amt/Unit	Total/Kit
Soluble Cytoplasmic Extraction Reagent	2	125 ml*	250 ml*
Soluble Protein Resuspension Reagent	1	23 ml*	23 ml*
Cellular and Organelle Membrane Solubilizing Reagent	1	23 ml*	23 ml*
Tributylphosphine Stock Solution	5	0.5 ml	2.5 ml
Alkylating Reagent, Iodoacetamide	5	56 mg	280 mg

*Represents reconstituted volume

Protein Extraction for Proteomics



Fraction 1

Fraction 2

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

ProteoPrep™ Reduction and Alkylation Kit

PROT-RA 1 kit

2-8°C

NEW

The ProteoPrep Reduction & Alkylation kit contains five vials of each reagent, which are used for reduction and alkylation of protein disulfide bonds. The reducing agent is Tributylphosphine (T 7567) and the alkylating reagent is Iodoacetamide (A 3221).

Features and Benefits

- Tributylphosphine is supplied safely as a 200 mM solution in N-methyl-2-pyrrolidine
- Reduction and alkylation of protein samples increase 2D spot resolution
- Conveniently packaged components save time and increase efficiency
- Compatibility with chaotropic extraction reagents simplifies sample preparation
- Comprehensive technical bulletin and optimized protocols save time and facilitate successful sample preparation

Shelf life minimum1 yr (when stored at 2-8°C)

R: 25-36/38-42/43 S: 26-36/37/39-45

Product Code	Name	Ampules/ Kit	Amt/ Ampule	Total/ Kit
T 7567	Tributylphosphine Stock Solution	10	0.5 ml	5 ml
A 3221	Alkylating Reagent, Iodoacetamide	10	56 mg	560 mg

Without Reduction and Alkylation

Source, plasma, reduced; 1st dimension IEF 3-10, 2nd dimension 12% SDS-PAGE

With Reduction and Alkylation prior to 1st dimension

Source, plasma, reduced and alkylated; 1st dimension IEF 3-10, 2nd dimension, 12% SDS-PAGE

Protein Extraction for Proteomics

ProteoPrep™ Protein Precipitation Kit

PROTPR RT NEW — This kit contains reagents sufficient for precipitating fifty 1 ml samples. — 1 kit

Features and Benefits
- Ready-to-use reagents save you time and ensure consistency
- Room-temperature storage saves you precious cooler space
- A comprehensive techinical bulletin provides an optimized protocol

Shelf life minimum1 yr (when stored at room temperature)

Components:
Trichloroacetic Acid, 100% (6.1 N)
Deoxycholate, 0.2% solution
Wash Solution, 25% acetone solution
R: 11-35-66-67 S: 9-16-26-36/37/39-45

Protein Purification

FLAG Affinity Purification

ANTI-FLAG® M2-Agarose

A 2220 -20-0°C WET ICE — **from mouse** — 1 mL
Purified immunoglobulin, Buffered aqueous glycerol solution — 5 mL, 10 mL, 25 mL

Anti-FLAG® is purified murine IgG1 monoclonal antibody covalently attached to beaded agarose. Useful for purification or immunoprecipitation of FLAG fusion proteins. Binds to the FLAG epitope wherever it is located in the fusion protein: amino-terminal, Met-amino-terminal, carboxy-terminal, or internal. Binding is not Ca^{2+}-dependent.
Affinity Gel (Freezer safe)
Suspension in buffered saline containing azide as preservative and 50% glycerol
Clone M2
Isotype. IgG1
binding capacity (FLAG-BAP). . . . . >0.6 mg/mL

EZview™ Red ANTI-FLAG® M2 Affinity Gel

F 2426 -20-0°C WET ICE — When performing small scale affinity capture, such as immunoprecipitation, the affinity matrix is difficult to see in the microcentrifuge tubes. Accidental aspiration of the resin leads to quantitative variability in results. The EZview™ Red Affinity Gels greatly reduces the risk of pellet loss. EZview™ resins perform as well as conventional non-colored affinity gels, but allow the user to easily differentiate pellet from supernatant. This correlates to more accurate data because less protein is lost. — 1 mL, 5 × 1 mL

Immunoprecipitation of FLAG- and 3xFLAG-tagged fusion proteins

Features and Benefits
- Increased visibility - Red color reduces risk of incidental aspiration
- Improved recovery of target protein reduces the need to repeat due to accidental loss
- Higher reproducibility - More consistent yields

N-terminal, Met-N-terminal, C-terminal FLAG fusion proteins, 3xFLAG fusion proteins
1:1 (v/v) suspension in PBS containing 50% glycerol and 15 ppm Kathon
binding capacity . . . . . ≥0.6 mg/mL

ANTI-FLAG® High Sensivity, M2 coated 96-well plates

P 2983 — A convenient, ready to use, platform for the capture and detection of FLAG fusion proteins. The ANTI-FLAG M2 antibody is covalently attached to the surface through the Fc portion and can detect 1 ng FLAG fusion protein/well with a capacity of up to 300 ng/well. Suitable for screening for expression, study of protein:protein interactions and ELISA assays. Used to detect N-terminal, Met-N-terminal, internal and C-terminal FLAG and 3XFLAG fusion proteins. Manufactured under ISO 9002 in Sigma's GMP facility, ANTI-FLAG high sensitivity M2 coated multiwell plates utilize a flat bottom, polystyrene baseplate. 96-well, clear — 1 each, 5 each

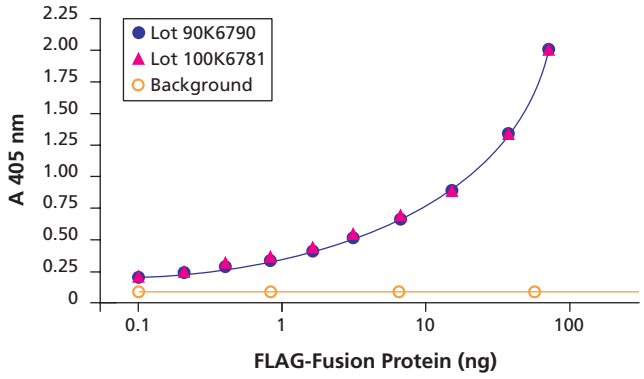


Figure 1. Consistent Sensitivity
Comparison of two lots of plates shows a high degree of well-to-well consistency (the error bars are too small to see under the data symbols). Sensitivity for both lots is well below 1 ng.

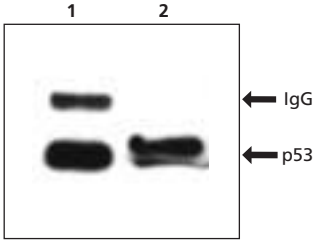


Figure 2. Western Blot of immunoprecipitated/captured FLAG-tagged p53.
FLAG-tagged p53 was immunoprecipitated/captured from cell lysates of COS-7 cells expressing FLAG-tagged p53.
1. p53 from ANTI-FLAG® M2 affinity gel was eluted by boiling in SDS-sample buffer.
2. p53 from ANTI-FLAG® plates was eluted with SDS buffer
The IgG band is absent (lane 2) with the ANTI-FLAG® M2 96-well plates even with harsh SDS elution. In contrast, significant amounts of IgG are released from affinity gel beads (lane 1).