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ProductInformation

ProteoPrep® Universal Extraction Kit

Product Code **PROT-TWO**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Sigma's ProteoPrep® Universal Extraction Kit is designed to separate the soluble cytoplasmic and membrane proteins from many types of cells. This kit also includes reagents for the reduction and alkylation of disulfide bonds. The cells are resuspended in a low conductivity buffer and disrupted by ultrasonication or other appropriate method. The membranes and membrane proteins are centrifuged and then washed once with buffer. The soluble protein fraction is dried and then resuspended with a chaotropic buffer. The insoluble material is resuspended and solublized in another chaotropic buffer solution. Both protein solutions are then reduced and alkylated. The two resulting soluble protein samples contain low salts and are ready for separation by isoelectric focusing (IEF), the first step in two-dimensional gel electrophoresis. This kit contains reagents sufficient to generate ten or more 2-ml samples.

Components

Soluble Cytoplasmic Extraction Reagent, 2 bottles of powder, each reconstitutes to 125 ml of 5 mM Trizma®, Product Code S 2688

Soluble Protein Resuspension Reagent, 1 bottle of powder that reconstitutes to a final volume of 23 ml of solution (pH 10.4) containing 7.0 M urea, 2.0 M thiourea, 1 % C7BzO detergent, Product Code S 3688

Protein Extraction Reagent Type 4, 1 bottle of powder that reconstitutes to a final volume of 23 ml of solution (pH 10.4) containing 7.0 M urea, 2.0 M thiourea, 1 % C7BzO detergent, 40 mM Trizma, Product Code C 0356

Tributylphosphine Stock Solution, 5 x 0.5 ml flame sealed ampules containing 200 mM tributylphosphine in N-methyl-2-pyrrolidone, Product Code T 7567

Alkylating Reagent, Iodoacetamide, 5 x 56 mg in brown glass vials, Product Code A 3221

Reagents and Equipment Required But Not Provided

- High purity water (Product Code W 4502)
- 37 °C water bath
- micropipettors
- graduated cylinder
- lyophilizer or SpeedVac®
- sonicator (e.g. Branson digital sonicator, model 450 or equivalent)
- centrifuge and centrifuge tubes

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reagents should be made fresh just prior to use as described below. As discussed below, unused quantities of some kit components can be frozen for multiple uses.

Soluble Cytoplasmic Extraction Reagent - Add 125 ml of high purity water to the contents of the container. Mix until dissolved. Unused material may be stored at 2 °C to 8 °C for up to 3 days. For longer storage, aliquots can be frozen at –20 °C for up to 6 months.

Soluble Protein Resuspension Reagent - Add 15 ml of high quality water to the container. This solution will become cold to the touch and needs to be warmed to 20-25 °C to dissolve completely. A 30 °C water bath will aid in the dissolution of the material. **Do not allow the temperature of the material to rise above 30 °C, since this product may begin to form cyanates that will be detrimental to the proteins.** Aliquot the unused material in 1 to 2 ml volumes and freeze at –20 °C for up to 6 months.

Protein Extraction Reagent Type 4 - Add 15 ml of high quality water to the contents of the container. This solution will become cold to the touch and needs to be warmed to 20-25 °C to dissolve completely. A 30 °C water bath will aid in the dissolution of the material. **Avoid storing the material above ambient temperatures since this product may begin to form cyanates that will be detrimental to the proteins.** Aliquot the unused material in 1 to 2 ml volumes and freeze at –20 °C for up to 6 months.

Tributylphosphine (TBP) Stock Solution - This reagent is a ready-to-use solution stored under argon in a flame sealed ampule. This stock solution is diluted 1:40 into the protein sample (25 µl of TBP stock solution into 1 ml of sample). Once the ampule is opened, the unused material should be quickly placed into an airtight glass vial, flushed with inert gas and kept at –20 °C for up to 2 weeks.

Alkylating Reagent, Iodoacetamide (IAA) - Dissolve the contents of one vial of Product A 3221 with 0.6 ml of high purity water. This will make a 0.5 M stock solution. Adding 30 µl of this stock solution to every 1 ml of sample results in a 15 mM IAA solution. For longer storage the material can be stored at –20 °C for up to 2 weeks.

Storage/Stability
These reagents should remain stable for at least 1 year in their unopened containers.

Procedure
Generalized procedure using *E. coli* as the cell type.

1. Suspend 20 mg of lyophilized *E. coli* (Product Code EC-1, strain K12) in 10 ml of Soluble Cytoplasmic Extraction Reagent (S 2688).
2. Sonicate this suspension on ice with the ultrasonic probe for one to two minutes to disrupt the cells and break down the DNA.
3. Centrifuge the suspension at approximately 14,000 x *g* for 45 minutes at 4 °C to pellet cell debris and insoluble protein material.
4. Decant and set the supernatant aside on ice.
5. To the pellet, add 10 ml of Soluble Cytoplasmic Extraction Reagent and repeat the sonication and centrifugation as above. This yields a supernatant and a pellet. Pool the supernatant with that from step 4, for a total soluble protein extract of approximately 20 ml (SUP1).
6. Lyophilize SUP1 overnight or dry in a vacuum centrifugation device (e.g. a SpeedVac).
7. Resuspend the lyophilized or dried soluble protein material in 2.0 ml of Soluble Protein Resuspension Reagent (S 3688). Store on ice until step 11.
8. Resuspend the cell pellet from step 5 in 2 ml of Protein Extraction Reagent Type 4 (C 0356), and sonicate to solubilize the proteins in this fraction (SUP2).
9. Centrifuge the suspension from step 8 at approximately 14,000 x *g* for 45 minutes at 15 °C to pellet cell debris.
10. Decant the supernatant (SUP2) from the centrifuge tube into a clean tube, discarding any residual pellet.
11. Reduce SUP1 and SUP2 separately by adding TBP to each to a final concentration of 5 mM (25 µl of TBP stock solution per ml solution) and incubate them for 1 hour at room temperature. **Do not add the alkylating reagent at this time as it will react with TBP.**
12. Alkylate SUP1 and SUP2 separately by adding iodoacetamide to each to a final concentration of 15 mM (30 µl of Alkylating Reagent, Iodoacetamide per ml solution) and incubate for 1.5 hours at room temperature.
13. Quench the remaining iodoacetamide with addition of TBP to a final concentration of 5 mM and incubate for another 15 minutes.
14. After the incubation, centrifuge the two reduced and alkylated samples at approximately 20,000 x *g* for five minutes at room temperature (microcentrifuge) to pellet any insoluble material.
15. SUP1 and SUP2 are now ready for loading onto IEF gels. Both samples may need to be diluted further with Protein Extraction Reagent Type 4 (C 0356) to obtain the desired results by two-dimensional gel electrophoresis. It is also suggested that the protein concentration be measured so that the amount of protein loaded onto the gels is known. The Bradford (Product code B 6916) assay is recommended for use with ProteoPrep kits; it has been found to be more accurate than either the BCA or Lowry assays.

Conversion for other sample types
Samples other than *E. coli* can be prepared with this procedure. Amounts of wet cell paste or tissue may be adjusted to fit the scale of the extraction. Use the following information only as a guideline.

The disruption of the cells depends upon the cell type. Yeast cells require a much more vigorous disruption using a bead mill, while tissue and mammalian cells may only require disruption by homogenization or simple blending.

Use a minimum of 2 ml of reagent per:
Wet cell paste (any species) = 50 to100 mg
Tissue = 250 mg

Protease inhibitors or protease inhibitor cocktails may be necessary to preserve the protein profile of certain samples. It may also be necessary to add nucleases to reduce the viscosity of the samples due to high molecular weight DNA. Addition of non-specific nucleases may help.[2] The two reagents containing chaotropes (Soluble Protein Resuspension Reagent and Protein Extraction Reagent Type 4) will denature most enzymes, so it is best to add any enzymes prior to their use.

Results

This kit generates two protein samples ready for application to immobilized pH gradient (IPG) strips for two-dimensional electrophoresis. These two fractions are enriched for soluble proteins (SUP1) and membrane proteins (SUP2). The membrane fraction from this separation will be different from that of the ProteoPrep Membrane Isolation Kit (Product Code PROT-MEM). The membrane fraction of the ProteoPrep Universal Extraction Kit contains membrane-associated proteins and some abundant cytoplasmic proteins in the membrane fraction. There may some hydrophobic proteins that will be found in both the membrane and the soluble cytoplasmic protein fractions. In contrast, the ProteoPrep Membrane Extraction Kit yields a membrane protein fraction containing only integral membrane proteins. The Membrane Extraction Kit utilizes a sodium carbonate extraction that removes nearly all of the membrane-associated proteins thus creating a classical membrane protein fraction. In samples more complex than *E. coli* such as yeast, insect, or mammalian cells and tissue, the nuclear and organelle proteins will be divided between these soluble and membrane fractions.

Related Products	**Product Code**
ProteoPrep® Kits	
Total Extraction Sample	PROT-TOT
Membrane Protein Extraction	PROT-MEM
Universal Extraction	PROT-TWO
Protein Extraction Reagent Type 4	C 0356
Protein Extraction Reagent Type 1	C 0481
Protein Extraction Reagent Type 2	C 0606
Protein Extraction Reagent Type 3	C 0731
ProteoGel™ IPG Strips	
pH 3-10	
7 cm	I 2531
11 cm	I 3406
18 cm	I 4031
pH 4-7	
7 cm	I 2906
11 cm	I 3531
18 cm	I 4156
pH 3-5	
7 cm	I 3031
11 cm	I 3656
18 cm	I 4281
pH 5-8	
7 cm	I 3156
11 cm	I 3781
18 cm	I 4406
pH 6-11	
7 cm	I 7406
11 cm	I 7531
18 cm	I 7656
pH 8-12	
7 cm	I 3281
11 cm	I 3906
18 cm	I 4531
IPG Equilibration Buffer	I 7281
EZBlue™ Gel Staining Reagent	G 1041
ProteoSilver Plus Staining Kit	PROT-SIL2
Bradford Reagent (recommended for 1-1,400 µg/ml protein)	B 6916
ProteoMass™ MALDI-MS Calibration Kits	
Protein and Peptide	MS-CAL1
Peptide	MS-CAL2
Protein	MS-CAL3
Protease Inhibitor Cocktail for General Use	P 2714
Protease Inhibitor Cocktail for Plant and Tissue Extracts	P 5955
Protease Inhibitor Cocktail for Fungal and Yeast Extracts	P 8215
Protease Inhibitor Cocktail for Mammalian Cell and Tissue Extracts	P 8340

References

1. Molloy, M.P., et al., Electrophoresis, **19,** 837-844 (1998).
2. Herbert, B.R., Electrophoresis, **19,** 845-851 (1998).

Technology developed in partnership with Proteome Systems™

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