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ProductInformation

ProteoPrep® Membrane Extraction Kit

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

TECHNICAL BULLETIN

Product Description

Sigma's ProteoPrep® Membrane Extraction Kit is designed to prepare a highly enriched membrane protein solution from many types of cells. This kit also includes reagents for the reduction and alkylation of disulfide bonds. The cells are suspended in a low conductivity buffer and disrupted by ultrasonication or other appropriate method. The membranes and membrane proteins are centrifuged, washed with water and then resuspended in a chaotropic buffer solution. This protein solution is then reduced and alkylated. The end result is a soluble membrane protein sample that contains low salt and is ready for separation on isoelectric focusing (IEF), the first step in two dimensional gel electrophoresis. This kit contains enough reagents to generate at least six 2 ml samples.

Components

Soluble Cytoplasmic and Loosely-bound Membrane Protein Extraction Reagent, 3 bottles of powder, each reconstitutes to 125 ml, Product Code S 2813

Protein Extraction Reagent Type 4, 1 bottle of powder that reconstitutes to a final volume of 23 ml, Product Code C 0356

Tributylphosphine Stock Solution, 5 x 0.5 ml flame sealed ampules, 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)
- 30 °C water bath
- micropipettors
- graduated cylinders
- 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 each use as described below. For most of the kit components, the unused solutions may be frozen for multiple uses.

Soluble Cytoplasmic and Loosely-bound Membrane Protein Extraction Reagent - Add 125 ml of high purity water to the contents of the container. Mix until all of the solid has gone into solution. Any unused reagent may be stored at 2-8 °C for up to 3 days. For longer storage, freeze the material at –20 °C.

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 for the entire solid to go into solution. A 30 °C water bath will aid in the dissolution of the material. **Do not allow the material to get above 30 °C since this product may begin to form cyanates that will be detrimental to the proteins.** Aliquot the unused material in 1-2 ml volumes and freeze at –20 °C for further use.

Tributylphosphine (TBP) Stock Solution - This reagent is a ready-to-use 200 mM TBP solution in N-methyl-2-pyrrolidone stored under argon in a flame sealed ampule. Once the ampule is opened the unused material may be stored up to 2 weeks if placed into an airtight glass vial and kept at –20 °C. This stock solution must be diluted 1:40 into the protein sample (50 µl of TBP stock solution into 2 ml of sample).

Alkylating Reagent, Iodoacetamide - Dissolve the contents of one vial of Product A 3221 with 0.6 ml of high purity water. Mix well until the entire solid has dissolved. This will make a 0.5 M stock solution. 60 µl of this stock solution should be added to every 2 ml of sample. Discard any remaining stock solution since it degrades quickly.

Storage/Stability
These reagents should remain stable for at least 1 year in their unopened container when stored properly at 2–8 °C.

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

1. Suspend 20 mg of lyophilized *E. coli* (Sigma product EC-1, strain K12) in 10 ml of ice cold Soluble Cytoplasmic and Loosely-bound Membrane ProteinExtraction Reagent (S 2813).
2. Using an ultrasonic probe, sonicate this suspension on ice for one to two minutes to disrupt the cells and break down the DNA.
3. Add 50 ml of ice cold Soluble Cytoplasmic and Loosely-bound Membrane Protein Extraction Reagent (S 2813).
4. Stir the suspension slowly on ice for 1 hour.
5. Ultracentrifuge the suspension (115,000 x *g* for 1 hour at 4 °C) to pellet membranes and membrane proteins.
6. Discard the supernatant. Resuspend the membrane pellet and wash it twice with 2 ml (each) of milliQ water (with centrifugation at 20,000 x *g* for 20 min at 4 °C). Note: The pellet must be thoroughly resuspended during these washes to remove all residual proteins and salts.
7. Resuspend the cell pellet in 2 ml of Protein Extraction Reagent Type 4 (C 0356) and sonicate on ice (70 % for 15 secs x 4) with the ultrasonic probe (Branson digital sonicator, model 450 or equivalent). Chill the suspension on ice between each of the four ultrasonication steps.
8. Centrifuge the suspension (14,000 x *g* for 45 minutes at 15 °C) to pellet cell debris. Decant the supernatant from the centrifuge tube into a clean tube.
9. Reduce this fraction by adding 50 µl TBP for each 2 ml of protein solution (TBP C_f=5 mM). Incubate at room temperature for 1 hour.
10. Alkylate this fraction by adding 60 µl of Alkylating Reagent, Iodoacetamide for each 2 ml of protein solution (Iodoacetamide C_f=15 mM). Incubate for 1.5 hours at room temperature.
11. Centrifuge the final reduced and alkylated sample at 20,000 x *g* for five minutes at room temperature (microcentrifuge) to pellet any insoluble material.
12. This material is now ready for loading onto IEF gels. This sample may need to be diluted further with Protein Extraction Reagent Type 4 (C 0356) to obtain the desired two dimensional gel results. The protein concentration should be measured so that the amount of protein loaded onto the gels is known.

Conversion for other sample types
Other sample types may be used with this procedure. The amount of input material may be adjusted to fit the scale of the extraction. Use the following information only as a guideline for starting. It may be easier to use more material per volume of extraction reagent since it can be diluted out at the end of the procedure. The disruption of the cells depends upon the cell type. Yeast cells require a much more vigorous disruption using a bead mill, while mammalian tissue or cells may only require disruption by homogenization or simple blending.

Use a minimum of 2 ml reagent per:
Wet cell paste (any species) = 50 to 100 mg
Tissue = 250 mg

Protease inhibitors or protease inhibitor cocktails may be necessary to preserve the protein profile of certain samples.

Protease Inhibitor Cocktail for:	**Prod. Code**
General Use	P 2714
Tissue Culture Media	P 1860
Bacterial Cell Extracts	P 8465
Fungal and Yeast Extracts	P 8215
Mammalian Cell and Tissue Extracts	P 8340
Plant Cell and Tissue Extracts	P 9599
Use in Purification of Histidine-tagged Proteins	P 8849

It may also be necessary to add nucleases to reduce the viscosity of the samples due to high molecular weight DNA.[2]

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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