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ProductInformation

ProteoPrep® Sample Extraction Kit

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

TECHNICAL BULLETIN

Product Description

Sigma's ProteoPrep® Sample Extraction Kit is designed to extract all of the proteins from any type of cell or tissue. It includes two reagents that have been used extensively for extraction and two new reagents with increased solubilizing power to extract more proteins. This kit also includes reagents for the reduction and alkylation of disulfide bonds. The cells are resuspended in any of the four chaotropic reagents provided and disrupted by ultrasonication or other appropriate method. The extract is clarified and the protein solution is then reduced and alkylated. The end result is a soluble total protein sample that contains low salt that 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 ten 2-ml samples using all four reagents.

Components

Protein Extraction Reagent Type 1
(CHAPS/Urea), 1 bottle of powder that reconstitutes to a final volume of 25 ml, Product Code C0481

Protein Extraction Reagent Type 2
(CHAPS/Urea/Thiourea/SB3-10), 1 bottle of powder that reconstitutes to a final volume of 25 ml, Product Code C0606

Protein Extraction Reagent Type 3
1 bottle of powder that reconstitutes to a final volume of 25 ml, Product Code C0731

Protein Extraction Reagent Type 4
1 bottle of powder that reconstitutes to a final volume of 23 ml, Product Code C0356

Tributylphosphine Stock Solution
5 x 0.5 ml flame sealed ampules, Product Code T7567

Alkylating Reagent, Iodoacetamide
5 x 56 mg in brown glass vials, Product Code A3221

Reagents and Equipment Required But Not Provided

- High purity water (Product Code W4502)
- 37 °C water bath
- micropipettors
- graduated cylinder
- sonicator (e.g. Branson digital sonicator, model 450 or equivalent)
- centrifuge and centrifuge tubes

Precautions and Disclaimer

Samples containing high amount of salts and buffers may not work with this kit. These samples should be dialyzed prior to use.

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. For most of the kit components, the unused solutions can be frozen for multiple uses.

Protein Extraction Reagent Type 1 - Add 17 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 37 °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.

Protein Extraction Reagent Type 2 - 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 37 °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.

Protein Extraction Reagent Type 3 - 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 37 °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.

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 37 °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 solution stored under argon in a flame sealed ampule. Once the ampule is opened the unused material can 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. Resuspend 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 material since it degrades quickly once it has been dissolved.

Storage/Stability

These reagents should remain stable for at least 6 months in their unopened container.

Procedure

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

1. Suspend 10 mg of lyophilized *E. coli* (Product Code EC-1, strain K12) in 2 ml of reagent 1,2,3 or 4 and sonicate on ice (4x 15 seconds) using an ultrasonic probe. 80 to 90 % of the dry weight of the *E. coli* is extractable protein. The temperature of the solution during sonication should not be allowed to rise above 30 °C. The temperature should also not fall below about 15 °C since the urea and thiourea will precipitate out of solution.
2. Centrifuge the suspension at 15,000 x *g* for 30 minutes at 15 ºC.
3. Decant the supernatant into a clean tube and discard the insoluble pellet.
4. Reduce the supernatant by adding TBP to a final concentration of 5 mM (50 µl of TBP stock solution) and incubating at room temperature for 1 hour.
5. Alkylate this solution by adding iodoacetamide to a final concentration of 15 mM (60 µl of Alkylating Reagent, iodoacetamide) and incubating for 1.5 hours at room temperature.
6. Centrifuge the final reduced and alkylated sample at 20,000 x *g* for five minutes at room temperature (microcentrifuge) to pellet any insoluble material.
7. This material is now ready for loading onto IEF gels. This sample may need to be diluted further with the extraction reagent. It also suggested that the protein concentration be measured so that the amount of protein loaded onto the gels is known.

Conversion for other sample types

Other sample types can be used with procedure. The amount of input material may be adjusted to fit the scale of the extraction. Use the following information only as a guideline. 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 tissue and mammalian 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. 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]

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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