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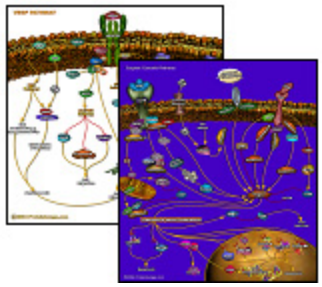


ProteoExtract® Protein Precipitation Kit
Cat. No. 539180

Note that this user protocol is not lot-specific and is representative of the current specifications for this product. Please consult the vial label and the certificate of analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions. Full details are available at www.calbiochem.com.

Size
1 kit

Form
200 Precipitations.

Storage
Upon arrival, all components of the kit can be stored at room temperature (20°C). For ease of use, the prepared Precipitation Agent and the Wash Solution can be stored at -20°C (see Reagent Preparation).

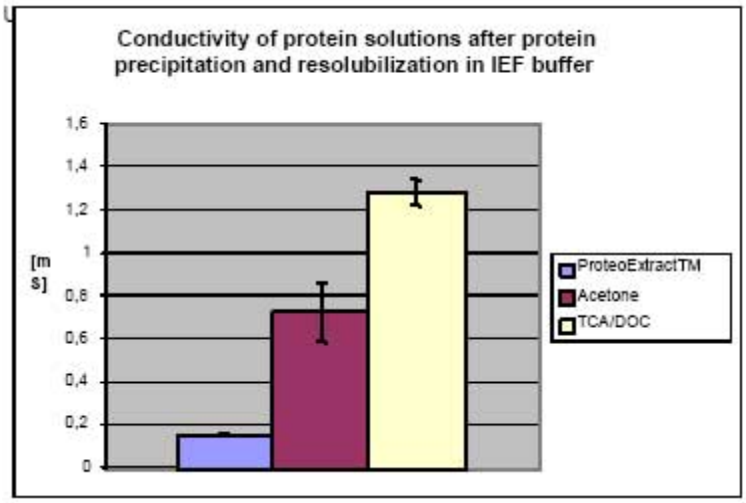
Intended Use
The ProteoExtract® Protein Precipitation Kit is designed for the concentration and clean-up of proteins from aqueous samples.

Background
Proteomics sample analysis is often hampered by the presence of non-protein impurities such as buffers, salts, and detergents that interfere with electrophoretic seperations or enzymatic digestion. Additionally, proteins in solution are often too dilute for direct downstream applications. Precipitation of proteins is procedure that can both concentrate proteins and remove interfering substances in one step.

Principle of the Assay
The ProteoExtract® Protein Precipitation Kit provides a fast and efficient method for concentrating and cleaning up proteins from a variety of sources. Detergents, chaotropic buffer components, salts, and other interfering compounds remain in the solution, while proteins are precipitated. Precipitated proteins can easily be re-suspended in the buffer of choice for a wide range downstream applications, such as isoelectric focusing (IEF), 2DGE, and 1DGE, or for tryptic digestion prior to mass spectrometry and peptide separation. The kit can be used with virtually any aqueous protein sample with a protein concentration ranging from 50 µg/ml to 10 mg/ml. Sample recovery is consistently higher then 90%. One kit is sufficient for the concentration and clean-up of 200 samples of 200 µl each. The procedure is scalable with sample volumes smaller or larger than 0.2 ml can be processed.

The ProteoExtract®; Protein Precipitation Kit yields protein solutions with very low conductivity compared to standard protein precipitation methods, making them ideally suited for isoelectric focusing (IEF), 2DGE, and 1DGE. The procedure does not result in selective loss of small proteins or changes in protein pattern relative to the untreated samples. ProteoExtract™ precipitated proteins are fully compatible with mass spectrometry (MS) following 2DGE separation as demonstrated by protein precipitation after removal of abundant proteins from human serum as model experiment.

Figure 1: Conductivity Following Protein Precipitation



The ProteoExtract® Protein Precipitation Kit yields protein samples with significantly lower conductivity then other precipitation methods.
Proteins at a concentration of 1 mg/ml were precipitated from 1 M sodium chloride solutions according to the Detailed Protocol. The same experiment was performed using standard protocols as TCA/DOC or acetone precipitation. Protein pellets were solubilized in IEF buffer for conductivity measurement.

Materials Provided
There are sufficient reagents to process 200 samples of 200 µl each.

- **Precipitant 1*:** 5 bottles; 29 ml each
- **Precipitant 2:** 1 bottle, 10 ml
- **Precipitant 3:** 1 bottle, 10 ml
- **Precipitant 4:** 1 bottle, 10 ml
- **Wash Solution*:** 1 bottle, 65 ml

*Precipitant 1 and the Wash Solution need to be reconstituted as described in the Reagent Preparation section.

Materials Required but not Provided
- Ethanol
- Microcentrifuge
- Micropipettes and tips, 10 µl, 200 µl and 1 ml size

Precautions and Recommendations
1. Always place the centrifuge tube in the centrifuge in the same orientation during the entire procedure so that the pellet remains on the same side of the tube after each centrifugation. This will minimize loss of protein pellets during centrifugation and washing.
2. The protocol can be applied to any volume, but optimization is required for protein precipitation from samples larger or smaller than 200 µl.

Sample Preparation

• **Samples:** The assay will precipitate proteins from a broad range of sample types containing from 50 µg/ml to 10 mg/ml protein. The assay will tolerate chaotropes (e.g., UREA) to to a concentration of 8 M, detergents up to 4%, and salts up to 2 M.

Reagent Preparation

Note: Prior to using the kit for the first time, the Precipitation Agent and the Wash Solution must be prepared and chilled to -20°C.

• Precipitation Agent:

1. To one bottle Precipitant 1 add 1.7 ml Precipitant 2, 1.7 ml Precipitant 3, and 1.7 ml Precipitant 4; mix well.
2. The mixture of Precipitant 1, 2, 3, and 4 is called the Precipitation Agent. One bottle Precipitation Agent (34.1ml) is sufficient for precipitation of 40 samples of 200 µl each.
3. Label the Precipitant 1 bottle after reconstitution for easy recognition.

• For ease of use, store the Precipitation Agent at -20°C; reconstituted Precipitation Agent is stable for up to 2 months.

• Wash Solution:

1. Add 150 ml high quality ethanol (not provided) to the bottle of Wash Solution (denatured ethanol may also be used).
2. Mix well and store at -20°C. Reconstituted Wash Solutions is stable for up to 1 year.

Note: Each 200 µl sample requires 800 µl Precipitation Agent and 1 ml Wash Solution.

Detailed Protocol

Note: The following protocols have been successfully used to precipitate and re-solubilize proteins from samples with a concentration range of 50 µg/ml to 10 mg/ml.

Protocol for Protein Precipitation from 200 µl Samples

1. In a microcentrifuge tube with conical bottom, mix 200 µl sample with 800 µl cold Precipitation Agent (-20°C). Vortex briefly.
2. Incubate for 20-60 min at -20°C. Longer incubation times will not affect the precipitation.

Note: A 60-min incubation period is recommended for very dilute samples.

3. Centrifute the sample for 10 min at room temperature, 10,000 g (14,000 rpm) to pellet the proteins.
4. Carefully aspirate the supernatant completely without disturbing the pellet.

Note: The pellet may be loose at this stage.

5. Wash the pellet by adding 500 µl cold Wash Solution (-20°C) and vortex briefly.
6. Centrifuge for 2 min at room temperature, 10,000 g (14,000 rpm) to pellet the proteins. Carefully aspirate the Wash Solution completely without disturbing the pellet.
7. Repeat steps 5 and 6.
8. Dry the pellet for 5 min to 1 h at room temperature by leaving the open tube on the lab bench (Note: the drying time will depend on the downstream application). Do not over-dry the pellet! Do not use a vacuum centrifuge. Over-drying the pellet will affect re-solubilization of the proteins.

Note: 5 min drying time is sufficient for tryptic digest and 1DGE sample preparation. 1 h drying time is recommended prior to re-solubilization in IEF buffer for 2DGE.

9. Dissolve the pellet in a buffer of choice (e.g., IEF buffer, tryptic digest buffer, or buffer compatible with 1DGE).

Protocol for Protein Precipitation from Samples Smaller than 200 µl

1. In a microcentrifuge tube with a conical bottom, mix the sample with four volume equivalents cold Precipitation

Agent (-20°C). Vortex briefly.
2. Incubate for 20-60 min at -20°C. Longer incubation times will not affect the precipitation.
Note: A 60-min incubation time is recommended for very dilute samples.
3. Centrifuge the tube for 10 min at room temperature, 10,000 g (14,000 rpm) to pellet the proteins.
4. Carefully aspirate the supernatant completely without disturbing the pellet.
Note: The pellet may be loose at this stage.
5. Add 500 µl cold Wash Solution (-20°C) and vortex briefly.
6. Centrifuge for 2 min at room temperature, 10,000 g (14,000 rpm) to pellet the proteins. Carefully aspirate the Wash Solution completely without disturbing the pellet.
7. Repeat steps 5 and 6
8. Dry the pellet for 5 min to 1 h at RT by leaving the open tube on the lab bench (Note: the drying time will depend on the downstream application). Do not over-dry the pellet! Do not use a vacuum centrifuge. Over-drying the pellet will affect re-solubilization of the proteins.
Note: 5 min drying time is sufficient for tryptic digest and 1DGE sample preparation. 1 h drying time is recommended prior to re-solubilization in IEF buffer for 2DGE.
9. Dissolve the pellet in a buffer of choice (e.g., IEF buffer, tryptic digest buffer, or buffer compatible with 1DGE).

Protocol for Protein Precipitation from Samples Larger than 200 µl

1. In a suitable centrifuge tube with conical bottom (e.g. 14 ml Falcon tubes) mix the sample with four volume equivalents of cold Precipitation Agent (-20°C). Vortex briefly.
2. Incubate for 20-60 min at -20°C. Longer incubation times will not affect the precipitation.
Note: A 60-min incubation time is recommended for very dilute samples.
3. Centrifuge the tube for 10 min at room temperature, 10,000 g (14,000 rpm) in a centrifuge that fits the tube.
4. Carefully aspirate the supernatant completely without disturbing the pellet.
Note: The pellet may be loose at this stage.
5. Add 1 ml cold Wash Solution (-20°C) and vortex briefly.
6. Resuspend the pellet and transfer to a clean microcentrifuge tube.
7. Centrifuge the tube for 2 min at room temperature, 10,000 g (14,000 rpm).
8. Carefully aspirate the Wash Solution completely without disturbing the pellet.
9. Add 500 µl cold Wash Solution (-20°C) and mix briefly.
10. Centrifuge the tube for 2 min at room temperature, 10,000 g (14,000 rpm).
11. Completely aspirate the Wash Solution without disturbing the pellet.
12. Dry the pellet for 5 min to 1 h at room temperature by leaving the open tube on the lab bench (Note: the drying time will depend on the downstream application). Do not over-dry the pellet! Do not use a vacuum centrifuge. Over-drying the pellet will affect re-solubilization of the proteins.
Note: 5 min drying time is sufficient for tryptic digest and 1DGE sample preparation. 1 h drying time is recommended prior to re-solubilization in IEF buffer for 2DGE.
13. Dissolve the pellet in a buffer of choice (e.g., IEF buffer, tryptic digest buffer, or buffer compatible with 1DGE).

Protocols for Preparation of Proteins for Downstream Analysis

Note: The method of choice for resolubilization of the precipitated proteins depends on the downstream application.

Resolubilization of Proteins for Two-Dimensional Gel Electrophoresis
• By thorough vortexing, resuspend the protein pellet in a suitable volume of your buffer of choice for isoelectric focusing (e.g. as provided with the Calbiochem® ProteoExtract™ Complete and Partial Proteome Extraction Kits. Alternatively a buffer comprising 7 M Urea, 2 M Thiourea, 50 mM DTT, 4% CHAPS has been found to be efficient.

• Incubate the sample at room temperature under gentle agitation for 1 h.
Notes: Do not heat the sample above 30°C, as protein carbamoylation may occur. A thermomixer is ideally suited for the incubation. If no thermomixer is available, please vortex the sample thoroughly every 15 min during the

incubation time.
• Centrifuge the sample for 5 min at room temperature, 10,000 g to remove any remaining insoluble material. Transfer the supernatant to a clean tube; the supernatant contains your protein sample. The concentrated and/or cleaned protein sample is now ready for isoelectric focusing.
• If desired, suitable ampholytes may be added to the sample prior to isoelectric focusing.

Resolubilization of Proteins for One-Dimensional Gel Electrophoresis

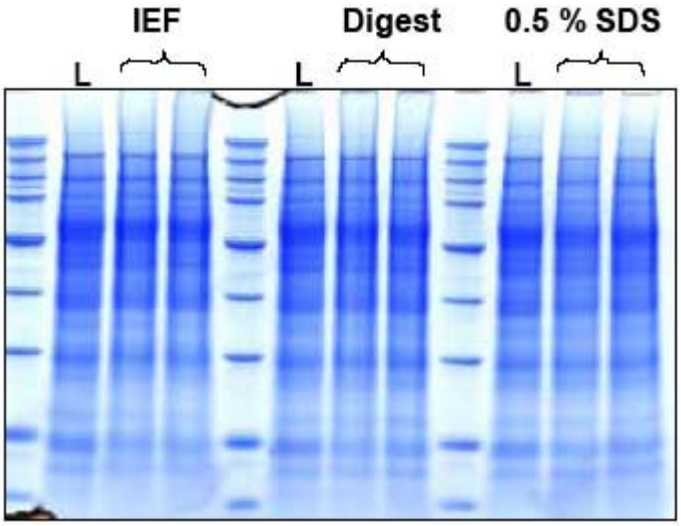
• By thorough vortexing, resuspend the protein pellet in a suitable volume of your buffer of choice for one-dimensional SDS-PAGE (e.g. SDS sample buffer, Cat. No. 70607-3). Alternatively, a buffer comprising 0.5% (w/v) SDS in 375 mM Tris-HCl, 0.5% (w/v) SDS, pH 8.8 has been found to be efficient.
• Heat the sample to 95°C for 10 min.
• Centrifuge the sample for 5 min at room temperature, 10,000 g to remove remaining insoluble material. Transfer the supernatant to a clean tube.
• The sample is now ready for 1D SDS-PAGE.

Resolubilization of Proteins for Tryptic Digestion

• By thorough vortexing, resuspend the protein pellet in a suitable volume of Digest Buffer provided with the ProteoExtract™ All-in-One Trypsin Digestion Kit, Cat. No. 650212.
• Incubate sample at room temperature under gentle agitation for 1 h. **Note:** Do not heat the sample above 30°C, as protein carbamoylation may occur. A thermomixer is ideally suited for the incubation. If no thermomixer is available, vortex the sample thoroughly every 15 min during the incubation time.
• Centrifuge the sample for 5 min at room temperature, 10,000 g to remove remaining insoluble material. Transfer the supernatant to a clean tube.
• The concentrated and/or cleaned protein sample is now ready for tryptic digestion.

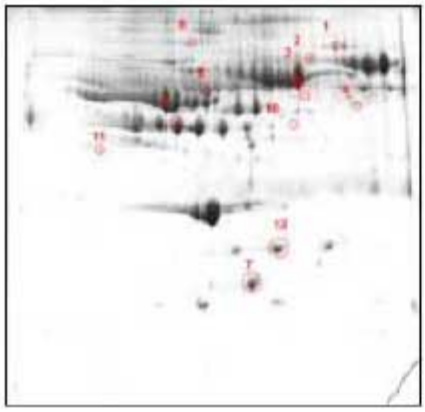
Example Data

Figure 2: Solubility Following Protein Precipitation



The ProteoExtract® Protein Precipitation Kit allows for the easy solubilization of the sample in standard buffers with near quantitative protein recovery. A liver extract prepared by lysis of tissue with 1% (w/v) SDS in 100 mM Tris-HCl, pH 7.0, at a concentration of 2 mg/ml was used to precipitate proteins according to the Detailed Protocol. Proteins were re-solubilized in IEF-buffer, tryptic digest buffer, or 0.5% SDS-buffer as outlined in the Detailed Protocol. L: crude liver extract in SDS; IEF: re-solubilized liver proteins in IEF-buffer; Digest: tryptic digest buffer; 0.5% SDS. Equal volume equivalents were loaded. The high protein yields without detectable protein loss clearly demonstrates the nearly quantitative sample recovery.

Figure 3: Compatibility With Mass Spectrometry and 2DGE



Spot	identity
1	complement Factor B Precursor
2	Gelsolin precursor
3	Human serum albumin
4	transferring n-terminal lobe
5	antithrombin III, chain L
6	haptoglobin chain beta
7	transthyretin
8	Inter-alpha trypsin inhibitor – related protein precursor
9	transferring fragment HUMTF-12 NID
10	apolipopretein L1 precursor
11	alpha-1-antitrypsin chain A
12	haptoglobin

The ProteoExtract® Protein Precipitation Kit is fully compatible with 2DGE and mass spectrometry. Human serum was processed to remove albumin and IgG using the ProteoExtract® Albumin/IgG Removal Kit, Cat. No. 122642. 1 mg protein was precipitated as outlined in the Detailed Protocol, resolubilized in IEF buffer, and analyzed by 2DGE. Selected spots were excised and proteins were digested using the ProteoExtract® All-in-One Trypsin Digestion Kit (Cat. No. 650212). Protein identities were obtained with a high level of statistical significance indicating that the precipitation procedure does not introduce any protein modifications that might interfere with mass spectrometry.

Troubleshooting

Problem: Protein loss
Possible Cause: Overdrying of protein pellets
Solution: Do not overdry the pellet or use a vacuum centrifuge as this will affect protein resolubilization.

Toxicity

MSDS available upon request.

Trademarks

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