ProteoExtract™ Protein Precipitation Kit

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Introduction

The ProteoExtract™ Protein Precipitation Kit is designed for concentration & clean up of proteins from aqueous samples.

Proteomics sample analysis is often hampered by the presence of non-protein impurities such as buffers, salts and detergents in the protein sample that interfere with electrophoretic separations or enzymatic digestion. Additionally, proteins in solutions are often too dilute for direct downstream applications such as 2DGE.
Precipitation of proteins is a protein purification procedure that can both concentrate the proteins and remove interfering substances in one step.

The ProteoExtract™ Protein Precipitation Kit provides a fast and efficient method to concentrate and clean up proteins from a variety of sources. Detergents, chaotropes buffer reagents, salts and other interfering compounds stay in solution. Precipitated proteins can easily be re-suspended in the buffer of choice for a wide range of proteomics downstream applications such as isoelectric focusing (IEF), 2DGE, 1DGE or for tryptic digestion prior to mass spectrometry and peptide separation schemes (Fig. 2).

The ProteoExtract™ Protein Precipitation Kit can be used with virtually any aqueous protein sample containing between 50 µg – 10 mg / ml protein. Sample recovery is consistently higher then 90%. One kit is sufficient for the concentration and clean up of 200 samples of 0.2 ml volume. The procedure is scalable, samples with volumes smaller or larger than 0.2 ml can be processed.

The ProteoExtract™ Protein Precipitation Kit delivers protein solutions with very low conductivity as compared to standard protein precipitation methods making them ideally suited for isoelectric focusing (IEF), 2DGE and 1DGE (Fig. 1). 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 (Fig. 3).

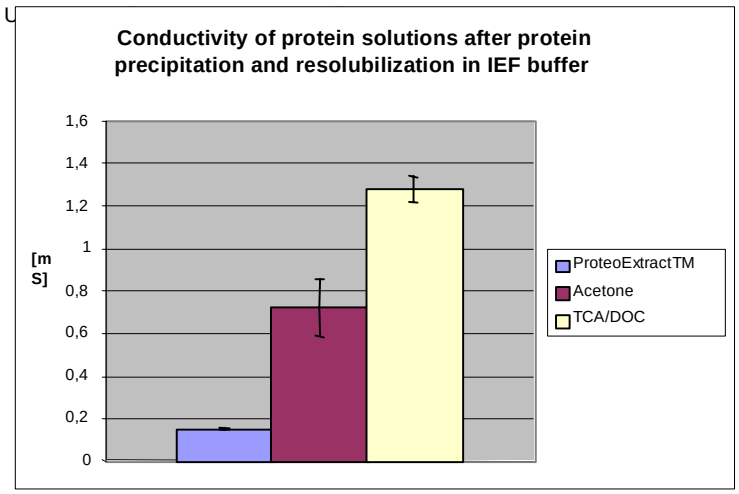


Fig.1 The ProteoExtract™ Protein Precipitation Kit delivers protein samples with significantly lower conductivity then other precipitation methods.
Proteins at a concentration of 1 mg/ml were precipitated from 1 M sodium chloride solutions using the ProteoExtract™ Protein Precipitation Kit. The same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

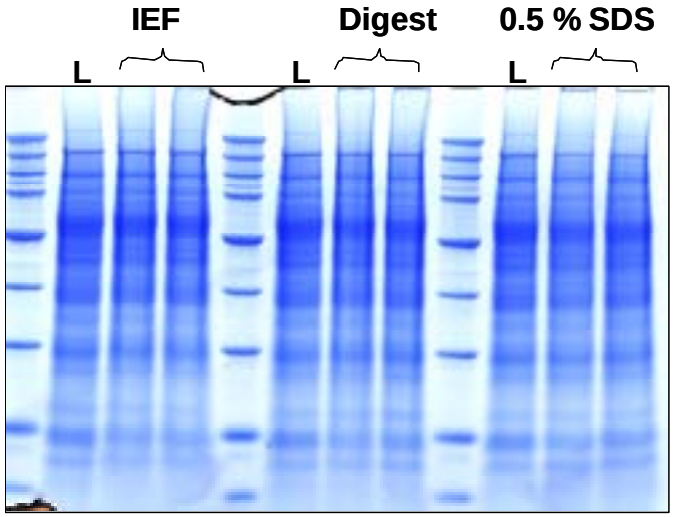
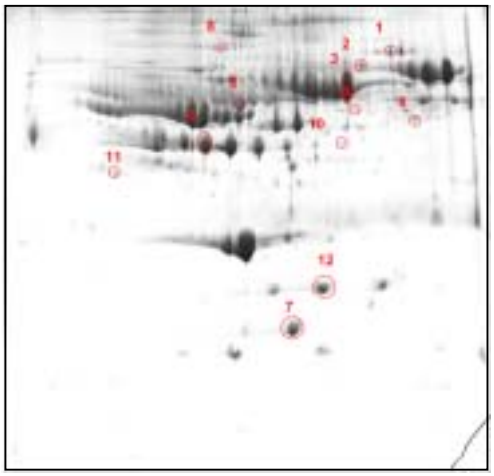


Fig.2 The ProteoExtract™ Protein Precipitation Kit allows for the easy solubilization of the sample in various standard buffers with near quantitative protein recovery.
A liver extract prepared by lysis of tissue with 1 % (w/v) SDS in 100 mM Tris/HCl, pH 7.0 at a protein concentration of 2 mg/ml was used as a model protein solution. Proteins were precipitated and re-solubilized in IEF-buffer, tryptic digest buffer or 0.5 % SDS-buffer as described in **9** followed by 1DGE. 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 losses clearly demonstrate the nearly quantitative sample recovery using the ProteoExtract™ Protein Precipitation Kit.



Spot	Identity
1	complement Factor B Precursor
2	Gelsolin precursor
3	human serum albumin
4	transferrin n-terminal lobe
5	antithrombin III, chain L
6	haptoglobin chain beta
7	transthyretin
8	inter-alpha trypsin inhibitor - related protein precursor
9	transferrin fragment HUMTF12 NID
10	apolipoprotein L1 precursor
11	alpha-1-antitrypsin chain A
12	haptoglobin chain alpha 2

Fig.3 The ProteoExtract™ Protein Precipitation Kit is fully compatible with 2DGE and mass spectrometry.

Human serum was subjected to albumin/IgG removal using the ProteoExtract™ Removal Kit (Cat. No. 122642). 1 mg protein was precipitated with the ProteoExtract™ Precipitation Kit, resolubilized in IEF buffer and subjected to 2DGE. Selected spots were excised and proteins digested using the ProteoExtract™ All-in-One Trypsin Digestion Kit (Cat. No. 650212). Protein identities were obtained with high statistical significance demonstrating that ProteoExtract™ Protein Precipitation Kit does not introduce any protein modifications that might interfere with mass spectrometry.

2. Kit Contents

Each kit contains sufficient reagents to process 200 samples of 200 µl each.

- Precipitant 1* 5 x 29 ml
- Precipitant 2 10 ml
- Precipitant 3 10 ml
- Precipitant 4 10 ml
- Wash Solution* 65 ml

*: Precipitant 1 and the Wash Solution need to be reconstituted as described below in **5.**

3. Storage Conditions

- All components of the kit can be stored at 20 – 25°C.

For convenience, we recommend to store Precipitation Agent and the Wash Solution at -20°C in order to have it ready-to-use.

4. Samples

Broad range of protein samples containing from 50 µg/ml – 10 mg/ml protein. Chaotropes as UREA up to 8 M; detergents up to 4%; salts up to 2 M.

5. Instructions for Preparation of Precipitation Agent and Wash Solution

Prior to use the Precipitation Agent and the Wash Solution have to be prepared and cooled to –20°C. (Please follow your facilities safety standards for handling flammable and potentially hazardous volatile compounds)

5.1. Preparation of Precipitation Agent

- To one bottle Precipitant 1 (= 29 ml) add 1.7 ml Precipitant 2, 1.7 ml Precipitant 3 and 1.7 ml Precipitant 4. Then mix well.
- The reconstituted Precipitant 1 is now referred as **Precipitation Agent.** One bottle of Precipitation Agent (34.1ml) is sufficient to precipitate 40 x 200 µl samples.
- Make a sign on the label of the Precipitant 1 of each bottle after reconstitution for easy recognition.
- For convenience store the Precipitation Agent (reconstituted Precipitant 1) at –20°C. It can be stored up to 2 months.

5.2. Preparation of Wash Solution

- Supplement the provided Wash Solution with 150 ml high quality ethanol (not provided). The ethanol may be denatured.
- Mix the reconstituted Wash Solution well and store at –20°C. It can be stored up to 1 year.

6. Kit Components Needed for One Extraction (200 µl Sample)

- 0.8 ml Precipitation Agent
- 1.0 ml Wash Solution

Reagents are sufficient for 200 samples of 200 µl (scalable).

7. Reagents and Equipment Not Provided

- Ethanol
- Microcentrifuge and rotor (Eppendorf, Heraeus or equivalent)
- Micropipettes and tips, 10 µl, 200 µl and 1 ml size (e.g., Eppendorf, Gilson or equivalent)
- Freezer

8. Protocols

General Guidelines

- The following protocols were successfully applied to precipitate and re-solubilize proteins in the concentration range between 50 µg/ml and 10 mg/ml.
- 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.
- Reconstitute the Precipitation Agent and the Wash Solution prior to assay.
- The protocol can be applied to any volume. Optimized protocols for protein precipitation from samples larger or smaller than 200 µl are given in sections 8.2 and 8.3.

8.1. Protein Precipitation from 0.2 ml Samples

1. In a microcentrifuge tube with conical bottom, mix 200 µl sample with 0.8 ml –20 °C cold Precipitation Agent. Vortex briefly.
2. Incubate 20 - 60 min at –20°C. Longer incubation times will not affect the result (a 60 min incubation time is recommended for very dilute samples).
3. Spin tube for 10 min at RT with 10.000 x g (14.000 rpm).
4. Carefully aspirate supernatant completely without disturbing the pellet. Note: The pellet may sometimes be loose at this stage.
5. Wash the pellet by adding 0.5 ml –20°C cold Wash Solution and vortex briefly.
6. Spin for 2 min at RT with 10.000 x g (14.000 rpm).
7. Carefully aspirate supernatant completely without disturbing the pellet.
8. Add 0.5 ml –20°C cold Wash Solution and vortex briefly.
9. Spin for 2 min at RT and 10.000 x g (14.000 rpm).
10. Completely aspirate the supernatant without disturbing the pellet.
11. Dry pellets for 5 min up to 1 hour at RT (depending on the further downstream application) by incubation of the open tube on the lab bench. Do not over-dry the pellet! Do not use a vacuum centrifuge. Over-drying the pellet will impair with re-solubilization of the proteins
    Note: 5 min drying are sufficient for tryptic digest and 1DGE sample preparation.
    1 h drying is recommended prior to re-solubilization in IEF buffer for 2DGE.
12. Dissolve the pellet in a buffer of choice for e.g. isoelectric focussing, tryptic digestion or 1DGE (see section 9.1 – 9.4).

8.2. Protein Precipitation from Samples Smaller than 0.2 ml

1. In a microcentrifuge tube with conical bottom, mix the sample volume with four volume equivalents of–20°C cold Precipitation Agent. Vortex briefly.
2. Incubate for 20 - 60 min at –20 °C. Longer incubation times will not affect the result (a 60 min incubation time is recommended for very dilute samples).
3. Spin tube for 10 min at RT and 10.000 x g (14.000 rpm).
4. Carefully aspirate supernatant completely without disturbing the pellet. Note: The pellet may sometimes be loose at this stage.
5. To wash the pellet, add 0.5 ml fresh –20°C cold Wash Solution and vortex briefly.
6. Spin for 2 min at RT and 10.000 x g (14.000 rpm).
7. Carefully aspirate supernatant completely without disturbing the pellet.
8. Add 0.5 ml fresh –20°C cold Wash Solution and vortex briefly.
9. Spin for 2 min at RT and 10.000 x g (14.000 rpm).
10. Completely aspirate the supernatant without disturbing the pellet.
11. Dry pellets for 5 min up to 1 h at RT by incubating the open tube on the lab bench.
    Note: 1 h drying is recommended prior to resolubilization in IEF buffer for 2DGE. 5 min drying time are sufficient for tryptic digest and 1DGE.

12. Important: Do not overdry the pellet. Do not use a vacuum centrifuge. Overdrying the pellet will impair with resolubilization of the proteins.
13. Dissolve the pellet in a suitable buffer of choice for e.g. isoelectric focussing, tryptic digestion or 1DGE (refer to section 9.1 – 9.4).

8.3. Protein Precipitation from Samples Larger than 0.2 ml

1. In a suitable centrifuge tube with conical bottom (e.g. 14 ml Falcon tubes) mix the sample volume with four volume equivalents of -20 °C cold Precipitation Agent. Vortex briefly.
2. Incubate for 20 to 60 min at –20°C. Longer incubation times will not affect the result (a 60 min incubation time is recommended for very dilute samples).
3. Spin tube for 10 min at RT and 10.000 x g (14.000 rpm) in a centrifuge that fits the sample tube.
4. Carefully aspirate supernatant completely without disturbing the pellet. Note: The pellet may sometimes be loose at this stage.
5. To wash the pellet, add 1 ml fresh –20°C cold Wash Solution and vortex briefly.
6. Transfer the pellet into a fresh microcentrifuge tube by resuspending it in the Wash Solution.
7. Spin microcentrifuge tube for 2 min at RT and 10.000 x g (14.000 rpm).
8. Carefully aspirate supernatant completely without disturbing the pellet.
9. Add 0.5 ml fresh –20 °C cold Wash Solution and vortex briefly.
10. Spin for 2 min at RT and 10.000 x g (14.000 rpm).
11. Completely aspirate the supernatant without disturbing the pellet.
12. Dry pellets for 5 min up to one hour at RT by incubation of the open tube on the lab bench. Note: One hour drying time is recommended prior to resolubilization in IEF buffer for two-dimensional gel electrophoresis. Five minutes drying time are sufficient for tryptic digest and 1DGE sample preparation.
    Important: Do not overdry the pellet. Do not use a vacuum centrifuge. Overdrying the pellet will impair with resolubilization of the proteins.
13. Dissolve the pellet in a suitable buffer of choice for e.g. isoelectric focussing, tryptic digestion or 1DGE.

9. Preparation of Proteins for Further Downstream Analysis

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

9.1. 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 focussing as e.g. provided with the Calbiochem ProteoExtract™ Complete and Partial Proteome Extraction Kits (see section 9: Related products). Alternatively a buffer comprising 7 M Urea, 2 M Thiourea, 4 % CHAPS and 50 mM DTT was found to be efficient.
- Incubate sample at RT under gentle agitation for one hour.
  Notes: Do not heat over + 30°C since protein carbamoylation may otherwise 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.
- Spin sample for 5 min at 10.000 x g and RT to remove remaining insoluble material. Collect supernatant.
- The concentrated and/or cleaned protein sample is now ready for isoelectric focussing.
- Optionally: Add suitable Ampholytes to sample prior to isoelectric focussing.

9.2. 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 as e.g. SDS sample buffer,
  Cat. # 70607-3, Novagen. Alternatively, a buffer comprising 0.5 % (w/v) SDS in 0.375 M Tris/HCl, pH 8.8 was found to be efficient.
- Heat sample to 95 °C for 10 min.
- Spin sample for 5 min at 10.000 x g and RT to remove remaining insoluble material. Collect supernatant.
- The concentrated and/or cleaned protein sample is now ready for 1 D-SDS-PAGE.

9.3. 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 RT under gentle agitation for one hour.
  Notes: Do not heat over + 30°C since protein carbamoylation may otherwise 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.
- Spin sample for 5 min at 10.000 x g and RT to remove remaining insoluble material. Collect supernatant.

The concentrated and/or cleaned protein sample is now ready for tryptic digestion

10. Troubleshooting

Problem	Possible Cause	Possible Remedy
Protein loss	Overdrying of protein pellets	Do not overdry pellets and do not use a vacuum centrifuge as this will impair with protein resolubilization.

11. Related Products

Ethanol : For the ethanol required for reconstitution of the Wash Solution we recommend pro analysi quality (Cat. No. 1.00974, Merck KGaA, Germany)
The ethanol may be denatured, e.g. with 1% ethylmethyl ketone without affecting the result.

	Cat. No.
ProteoExtract™ Kits	
Subcellular Proteome Extraction Kit	539790
Complete Bacterial Proteome Extraction Kit	539770
Complete Yeast Proteome Extraction Kit	539775
Complete Mammalian Proteome Extraction Kit	539779
Partial Bacterial Proteome Extraction Kit	539780
Partial Yeast Proteome Extraction Kit	539785
Partial Mammalian Proteome Extraction Kit	539789
Albumin Removal Kit	122640
Albumin/IgG Removal Kit	122642
All-in-One Trypsin Digestion Kit	650212
SDS Sample Buffer	70607-3

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