2-D Clean-Up Kit

Product Specification Sheet

Introduction

The 2-D Clean-Up Kit is designed to prepare samples for 2-D electrophoresis that otherwise produce poor 2-D results due to high conductivity, high levels of interfering substances or low concentration. The procedure works by quantitatively precipitating proteins while leaving behind in solution interfering substances such as detergents, salts, lipids, phenolics and nucleic acids. The proteins are then resuspended in a solution compatible with first dimension isoelectric focusing (IEF). The procedure can be completed in one hour and does not result in spot gain or loss. The kit contains sufficient reagents to process 50 samples of up to 100 µL each. The procedure can be scaled up for larger volumes or more dilute samples.

Intended use

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Storage

The kit should be stored at room temperature. The wash buffer should be placed at -20°C at least 1 h before use and may be stored in a -20°C freezer.

Function testing

Each lot of the 2-D Clean-Up Kit is tested for its ability to quantitatively precipitate protein and allow quantitative resuspension while minimizing carry-over of ionic contaminants.

Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/ or safety statement(s) for specific advice.

Components

Precipitant: This solution renders proteins insoluble.

Co-precipitant: This solution contains reagents that co-precipitate with proteins and enhances their removal from solution.

Wash buffer: This solution is used to remove non-protein contaminants from the protein precipitate.

Wash additive: This solution contains a reagent that promotes rapid and complete resuspension of the sample proteins.

Overview

The ability to analyze a sample effectively by 2-D electrophoresis often limited by the presence of non-protein impurities in the sample. Excess salts and buffers from sample preparation can render the solution too conductive for effective first dimension IEF. Charged detergents, lipids, phenolics and nucleic acids can also interfere both with first dimension IEF separation and visualization of the 2-D result. Protein precipitation is therefore often employed to selectively separate proteins in the sample from contaminating substances. Protein precipitation is also used to concentrate proteins from samples that are too dilute for effective 2-D analysis.

Current methods of protein precipitation suffer from several significant disadvantages.

1. Precipitation can be incomplete, resulting in the loss of proteins from the sample and introduction of bias into the 2-D result.
2. The precipitated protein can be difficult to resuspend and often cannot be fully recovered.
3. The precipitation procedure can itself introduce ions that interfere with first dimension IEF.
4. Precipitation can be time consuming, requiring overnight incubation of the sample.

The 2-D Clean-Up Kit provides a method for selectively precipitating protein for 2-D electrophoretic analysis that circumvents these disadvantages. Protein can be quantitatively precipitated from a variety of sources without interference from detergents, chaotropes and other common reagents used to solubilize protein. Recovery is generally above 90%. The procedure does not result in spot gain or loss, or changes in spot position relative to untreated samples. The precipitated proteins are easily resuspended in 2-D sample solution. The procedure can be completed in under one hour.

Treatment of the sample with the 2-D Clean-Up Kit can improve the quality of 2-D electrophoresis results, reducing streaking, background staining and other consequences of interfering contaminants. The kit can enable effective 2-D analysis of samples that are otherwise too dirty or dilute.

The 2-D Clean-Up Kit procedure uses a combination of a unique precipitant and co-precipitant to quantitatively precipitate the sample proteins. The proteins are pelleted by centrifugation and the precipitate is washed to further remove non-protein contaminants. The mixture is centrifuged again and the resultant pellet can be easily resuspended into a 2-D sample solution of choice.

Protocol

Introduction

The 2-D Clean-Up Kit can be used on virtually any protein sample. Lysis and extraction solutions that would normally be incompatible with first dimension IEF due to the presence of salts or charged detergents may be employed. These interfering substances are removed by the procedure.

If desired, sample proteins may be extracted from cells or solid tissues using the Sample Grinding Kit and a solubilizing solution consisting of 2% sodium dodecylsulfate (SDS), 40 mM Tris base and 60 mM dithiothreitol (DTT). The extract is heated at 95–100°C for 5 min and insoluble debris and grinding resin is removed by centrifugation.

The 2-D Clean-Up Kit can be used to prepare concentrated protein samples from sources that are dilute and contain high levels of salt and other interfering substances. Examples include urine, culture supernatants and plant extracts.

Proteases are generally inactive in the solutions employed in this procedure, but protease inhibitors can be added to the sample solution if desired.

The sample can contain 1–100 µg protein in a volume of 1–100 µL (**Procedure A**). Protein can be processed from larger samples by scaling up the procedure (**Procedure B**).

Hint: *Always position the microcentrifuge tubes in the centrifuge rotor with the cap hinge facing outward. This way the pellet will always be on the same side of the tube so it can be left undisturbed, minimizing loss.*

Required but not provided:

- 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of spinning tubes at 12 000 × g or more at 4°C
- Rehydration or sample solution for resuspension (See *Appendix: Rehydration of IEF sample loading solution on page 0* for examples)
- Vortex mixer

Preliminary preparations

Place the wash buffer at -20°C at least one h before starting the procedure. The wash buffer may be stored in a -20°C freezer.

The protein sample should be substantially free of particulate material. Clarify by centrifugation if necessary.

Procedure A

Procedure for sample volumes of 1–100 µL (containing 1–100 µg protein per sample)

Process the protein samples in 1.5 mL microcentrifuge tubes. All steps should be carried out with the tubes in an ice bucket unless otherwise specified.

Step	Action
1	Transfer 1–100 µL protein sample (containing 1–100 µg protein) into a 1.5 mL microcentrifuge tube.
2	Add 300 µL precipitant and mix well by vortexing or inversion. incubate on ice (4–5°C) for 15 min.
3	Add 300 µL co-precipitant to the mixture of protein and precipitant. Mix by vortexing briefly.
4	Centrifuge the tubes in a microcentrifuge set at maximum speed (at least 12 000 × g) for 5 min. Remove the tubes from the centrifuge as soon as centrifugation is complete. A small pellet should be visible. Proceed rapidly to the next step to avoid resuspension or dispersion of the pellet.
5	Remove as much of the supernatant as possible by decanting or careful pipetting. Do not disturb the pellet.
6	Carefully reposition the tubes in the microcentrifuge as before, with the cap-hinge and pellet facing outward. Centrifuge the tubes again to bring any remaining liquid to the bottom of the tube. A brief pulse is sufficient. Use a pipette tip to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
7	Without disturbing the pellet, layer 40 µL of co-precipitant on top of the pellet. Let the tube sit on ice for 5 min.
8	Carefully reposition the tube in the centrifuge as before, i.e. cap hinge facing outward. Centrifuge the tube again for 5 min. Use a pipette tip, remove and discard the wash.
9	Pipette 25 µL of distilled or de-ionized water on top of each pellet. Vortex each tube for 5–10 s. The pellet should disperse, but not dissolve in the water.
10	Add 1 mL of wash buffer (pre-chilled for at least 1 h at -20°C) and 5 µL wash additive. Vortex until the pellet is fully dispersed. ***Note:*** *The protein pellet will not dissolve in the wash buffer.*
11	Incubate the tubes at -20°C for at least 30 min. Vortex for 20–30 s once every 10 min. ***Note:*** *The tubes can be left at this stage at -20°C for up to 1 week with minimal protein degradation or modification.*
12	Centrifuge the tubes in a microcentrifuge set at maximum speed (at least 12 000 × g) for 5 min.
13	Carefully remove and discard the supernatant. A white pellet should be visible. Allow the pellet to air dry briefly (for no more than 5 min). ***Note:*** *Do not over-dry the pellet. If it becomes too dry, it will be difficult to resuspend.*
14	Resuspend each pellet in an appropriate volume of rehydration or IEF sample loading solution for first dimension IEF. See *Appendix: Rehydration of IEF sample loading solution on page 0* for examples of rehydration solutions and volumes appropriate to different applications. Vortex the tube for at least 30 s. Incubate at room temperature and either vortex or work up and down in a pipette to fully dissolve. ***Note:*** *If the pellet is large or too dry, it may be slow to resuspend fully. Sonication or treatment with the Sample Grinding Kit can speed resuspension.*
15	Centrifuge the tubes in a microcentrifuge set at maximum speed (at least 12 000 × g) for 5 min to remove any insoluble material and to reduce any foam. The supernatant may be loaded directly onto first dimension IEF or transferred to another tube and stored at -80°C for later analysis.

Procedure B

Procedure for larger sample of more than 100 µg protein

Procedure for larger samples of more than 100 µg of protein. All steps should be carried out with the tubes in an ice bucket unless otherwise specified.

Step	Action
1	Transfer the protein sample into a tube that can be centrifuged at 8 000 × g. The tube must have a capacity at least 12× greater than the volume of the sample. Use only polypropylene, polyallomer or glass tubes. ***Note:*** *The wash buffer used later in the procedure attacks many plastics. This limits the choice of centrifuge tube materials.*
2	For each volume of sample, add 3 volumes of precipitant. Mix well by vortexing or inversion. Incubate on ice (4–5°C) for 15 min.
3	For each original volume of sample, add 3 volumes of co-precipitant to the mixture of protein and precipitant. Mix by vortexing briefly.
4	Centrifuge the tubes at 8 000 × g for 10 min. Remove the tubes from the centrifuge as soon as centrifugation is complete. A small pellet should be visible. Proceed rapidly to the next step to avoid resuspension or diffusion of the pellet.
5	Remove as much of the supernatant as possible by decanting or careful pipetting. Do not disturb the pellet.
6	Carefully reposition the tubes in the centrifuge as before with the pellet facing outward. Centrifuge the tubes again for at least 1 min to bring any remaining liquid to the bottom of the tubes. Use a pipette tip to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
7	Add co-precipitant 3–4× times the size of the pellet.
8	Carefully reposition the tube in the cetrifuge as before, i.e. cap hinge facing outward. Centrifuge the tube again for 5 min. use a pipette tip, remove and discard the wash.
9	Pipette enough distilled or de-ionized water on top of each pellet to cover the pellet. Vortex each tube for several seconds. The pellets should disperse, but not dissolve in the water.
10	Add 1 mL of wash buffer, pre-chilled for at least 1 h at -20°C. (For initial protein sample in the range of 0.1–0.3 mL add 1 mL of wash buffer; in addition, the volume of wash buffer must be at least 10-fold greater than the water added in step 9). Add 5 µL wash additive (regardless of the original sample volume, use only 5 µL wash additive). Vortex until the pellet is fully dispersed. ***Note:*** *The protein pellet will not dissolve in the wash buffer.*
11	Incubate the tubes at -20°C for at least 30 min. Vortex for 20–30 s once every 10 min. ***Note:*** *The tubes can be left at this stage at -20°C for up to 1 week with minimal protein degradation or modification.*
12	Centrifuge the tubes at 8 000 × g for 10 min.
13	Carefully remove and discard the supernatant. A white pellet should be visible. Allow the pellet to air dry briefly (no more than 5 min). ***Note:*** *Do not over-dry the pellet. If it becomes too dry, it will be difficult to resuspend.*
14	Resuspend each pellet in rehydration solution for first dimension IEF. The volume of rehydration solution used can be as little as 1/20 the volume of the original sample. See *Appendix: Rehydration of IEF sample loading solution on page 0* for examples of rehydration solutions and volumes appropriate to different applications. Vortex the tube for 30 s. Incubate at room temperature and either vortex or work up and down in a pipette to fully dissolve. ***Note:*** *If the pellet is large or too dry, it may be slow to resuspend fully. Sonication or treatment with the Sample Grinding Kit can speed resuspension.*
15	Centrifuge the tubes at 8 000 × g for 10 min to remove any insoluble material and to reduce any foam. The supernatant may be loaded directly onto first dimension IEF or transferred to another tube and stored at -80°C for later analysis.

Appendix: Rehydration of IEF sample loading solution

The 2-D Clean-Up Kit produces a protein pellet that can be resuspended in sample or rehydration solution and applied directly to first dimension IEF (see step 12 of Procedures A and B).

The sample can be loaded into the Immobiline™ DryStrip gel (IPG strip) by rehydration or with a sample cup. In either case, the solution used is the same. If the sample is loaded using a sample cup, the sample loading solution and the rehydration solution (not containing protein) should have the same composition.

Examples of rehydration/IEF sample loading solutions:

Table 1. Rehydration solution containing 8 M urea

(8 M urea, 2% CHAPS, 40 mM DTT, 0.5% Pharmalyte™ or IPG buffer, 0.002% bromophenol blue, 2.5 mL). This is a good all-purpose solution that gives clean, sharp 2-D separations.

	final concentration	amount
Urea (FW 60.06)	8 M	1.20 g
CHAPS[1]	2% (w/v)	50 mg
Carrier ampholyte[2] (Pharmalyte or IPG Buffer)	0.5% (v/v)[3]	12.5 µL
DTT (FW 154.2)	40 mM	15.4 mg
Bromophenol Blue	0.002% (w/v)	5 µL of a 1% (w/v) solution
Distilled or de-ionized water		to 2.5 mL

[1] Other neutral or zwitterionic detergents may be used. Examples include NP-40, octyl glucoside and the alkylamidosulfobetaine detergents ASB-14 and ASB-16.

[2] Use IPG buffer in the pH range corresponding to the pH range of the IEF separation to be performed, or Pharmalyte in a pH range approximating the pH range of the IEF separation to be performed.

[3] Concentrations greater than 0.5% may be used for some applications. See "2-D Electrophoresis, Principles and Methods" for guidelines.

Table 2. Rehydration solution containing 7 M urea, 2 M thiourea

(7 M urea, 2 M thiourea, 4% CHAPS, 40 mM DTT, 0.5% Pharmalyte or IPG buffer, 0.002% bromophenol blue, 2.5 mL). This is a more strongly solubilizing solution that can result in more proteins solubilized and more spots in the final 2-D pattern. IEF separations performed with this solution may not be as sharp as with the previous solution, resulting in a 2-D separation with more horizontal streaking.

	final concentration	amount
Urea (FW 60.06)	7 M	1.05 g
Thiourea (FW 76.12)	2 M	381 mg
CHAPS[1]	4% (w/v)	100 mg
Carrier ampholyte[2] (Pharmalyte or IPG Buffer)	0.5% (v/v)[3]	12.5 µL
DTT (FW 154.2)	40 mM	15.4 mg
Bromophenol Blue	0.002% (w/v)	5 µL of a 1% (w/v) solution
Distilled or de-ionized water		to 2.5 mL

[1] Other neutral or zwitterionic detergents may be used. Examples include NP-40, octyl glucoside and the alkylamidosulfobetaine detergents ASB-14 and ASB-16.

[2] Use IPG buffer in the pH range corresponding to the pH range of the IEF separation to be performed, or Pharmalyte in a pH range approximating the pH range of the IEF separation to be performed.

[3] Concentrations greater than 0.5% may be used for some applications. See "2-D Electrophoresis, Principles and Methods" for guidelines.

Any other components added to the rehydration solution must either be uncharged or present at a concentration of less than 1 mM. Addition of salts, acids, bases and buffers is not recommended.

Larger quantities of rehydration solution may be prepared and stored in aliquots at -20°C or lower. If this is done, the DTT should be omitted and added fresh directly prior to use. The rehydration solution can be made in advance omitting carrier ampholyte. The IPG buffer or Pharmalyte appropriate to the IEF separation range is then added just prior to use.

The volume of solution used to resuspend the sample depends on the method of loading the sample and the length of the IPG strip used for the first dimension separation. If the sample is to be loaded onto the IPG strip using a sample cup, the sample volume should not exceed 100 µL. If the sample is to be loaded onto the IPG strip by rehydration, the following sample volumes should be used according to the length of the IPG strip.

IPG strip length	volume applied
7 cm	125 µL
11 cm	200 µL
13 cm	250 µL
18 cm	350 µL
24 cm	450 µL

Samples may be resuspended in volumes lower than the recommended application volume, if a more concentrated sample is desired. The sample would then be diluted appropriately into rehydration solution prior to application to the IPG strip.

The optimal quantity of protein to load varies widely depending on factors such as sample complexity, the length and pH range of the IPG strip and the method of visualizing the 2-D separation. General guidelines are given in "2-D Electrophoresis, Principles and Methods."

The protein concentration of the sample is best determined using the 2-D Quant Kit, which can accurately quantify protein in the presence of detergents, reductants and other reagents used in sample preparation.

Ordering information

Product	Quantity	Code No.
2-D Clean-Up Kit	50 samples	80-6484-51

Related products	Quantity	Code No.
Tris	500 g	17-1321-01
Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Dithiothreitol (DTT)	1 g	17-1318-01
Sodium Dodecylsulfate (SDS)	100 g	17-1313-01
Bromophenol Blue	10 g	17-1329-01
Sample Grinding Kit	50 samples, up to 100 mg tissue or cell sample	80-6483-37
2-D Quant Kit	500 assays, 1–50 µL and up to 50 µg	80-6483-56
SDS-PAGE Clean-Up Kit	50 samples, 1–100 µL	80-6484-70
Mini Dialysis Kit	1 kDa cut-off, up to 250 µL	80-6483-75
Mini Dialysis Kit	1 kDa cut-off, up to 2 mL	80-6483-94
Mini Dialysis Kit	8 kDa cut-off, up to 250 µL	80-6484-13
Mini Dialysis Kit	8 kDa cut-off, up to 2 mL	80-6484-32
2-D Protein Extraction Buffer Trial Kit	for 6 × 10 mL	28-9435-22
2-D Protein Extraction Buffer-I	for 50 mL	28-9435-23
2-D Protein Extraction Buffer-II	for 50 mL	28-9435-24
2-D Protein Extraction Buffer-III	for 50 mL	28-9435-25
2-D Protein Extraction Buffer-IV	for 50 mL	28-9435-26
2-D Protein Extraction Buffer-V	for 50 mL	28-9435-27
2-D Protein Extraction Buffer-VI	for 50 mL	28-9435-28
Nuclease Mix	0.5 mL	80-6501-42
Protease Inhibitor Mix	1 mL	80-6501-23
Vivaspin™ ultracentrifugation devices	Multiple	

Other reagents
Phenylmethyl Sulfonyl Fluoride (PMSF)[1]
Aprotinin[1]
Leupeptin[1]
Immobiline DryStrip[1]

Other reagents
Pharmalyte[1]
IPG Buffer[1]

[1] See the CytivaCatalogue for full selection

Related literature	Code No.
Handbook: 2-D Electrophoresis Using Immobilized pH Gradients, Principles & Methods.	80-6429-60

cytiva.com

For local office contact information, visit cytiva.com/contact

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