PlusOne SDS-PAGE
Clean-Up Kit
Product Specification Sheet

Introduction

Product code

80648470

Description

PlusOne SDS-PAGE Clean-Up Kit is designed to prepare samples for SDS-PAGE that are otherwise difficult to analyze due to high conductivity or low protein 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 and mixed with SDS-PAGE sample buffer. The procedure can be completed in under 2 hours with quantitative yield. 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.

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Function testing

Each lot of the PlusOne SDS-PAGE Clean-Up Kit is tested for its ability to quantitatively precipitate protein and allow quantitative resuspension.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

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.

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.

Buffer I:

Sample proteins are resuspended in this solution.

Buffer II:

This solution is added to the resuspended sample.

SDS-PAGE sample buffer:

This solution contains the remaining components necessary to prepare a sample for SDS-PAGE.

Overview

In order for protein samples loaded onto an SDS gel to yield clear, distortion-free bands of constant width, each sample must have the same buffer and ionic composition. Laboratory samples, however, often contain varying buffers, salts or detergents which can result in non-optimal resolution and lane distortions.

Many sample sources are too dilute to be directly treated and loaded on SDS gels. Such samples must be concentrated prior to SDS-PAGE analysis.

Selective precipitation of sample proteins, followed by resuspension in a small volume of SDS-PAGE-compatible sample buffer, can be employed to circumvent problems of interfering substances and differing electrolyte backgrounds among samples, as well as providing effective concentration of samples that are otherwise too dilute for SDS-PAGE analysis.

PlusOne SDS-PAGE Clean-Up Kit provides a method for selectively precipitating protein for SDS-PAGE analysis that is both rapid and quantitative. Protein can be precipitated from a variety of sources without interference from detergents, chaotropes and other

common reagents used to solubilize protein. Recovery is generally above 90% and the procedure results in minimal loss or modifi cation of proteins. The kit also contains reagents for resuspension of the sample in SDS-PAGE-compatible sample buffer. The entire procedure can be completed in under two hours.

Treatment of the sample with PlusOne SDS-PAGE Clean-Up Kit can improve the quality of SDS-PAGE results, reducing lane distortion, increasing resolution and yielding “publication quality” electrophoresis results. The kit can enable effective SDS-PAGE analysis of samples that are otherwise too dirty or dilute.

The PlusOne SDS-PAGE 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 is resuspended, mixed with SDS-PAGE sample buffer and heated. The sample is then ready to be loaded onto an SDS gel.

Protocol

Description

The PlusOne SDS-PAGE Clean-Up Kit can be used on virtually any protein sample, including those containing high concentrations of salts, detergents or denaturants that can render SDS-PAGE analysis diffi cult. Concentrated protein samples can also be prepared from sources as dilute as 1 ng/mL.

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 µg to 1 mg protein in a volume of 1 to 100 µL (**Procedure A**). Proteins can be processed from larger volumes by scaling up the procedure (**Procedure B**).

Tip: *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
- Vortex mixer
- 2-mercaptoethanol or dithiothreitol (DTT)
- Boiling water bath or heat block set at 95°C

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.

Add reductant to the SDS-PAGE sample buffer. The reductant can be either DTT or 2-mercaptoethanol. If using DTT, add 3.1 mg per 100 µL of SDS-PAGE sample buffer to be used. Make sure it is fully dissolved. If using 2-mercaptoethanol, add 5 µL per 100 µL of SDS-PAGE sample buffer to be used. Once the reductant has been added, the SDSPAGE sample buffer should be used immediately, so reductant should only be added to the amount of SDS-PAGE sample buffer needed for a single experiment.

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

Procedure A

for sample volumes of 1–100 µL

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

Step	Action
1	Transfer 1–100 µL protein sample (containing 1 µg to 1 mg protein) into a 1.5 mL microcentrifuge tube.
2	Add 300 µL precipitant (labeled with 1) and mix well by vortexing or inversion. Incubate on ice (4–5°C) for 15 min.
3	Add 300 µL co-precipitant (labeled with 2) to the mixture of protein and precipitant. Mix by vortexing briefl y.
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 suffi cient. Use a micropipette tip to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
7	Pipette 25 µL of distilled or de-ionized water on top of each pellet. Vortex each tube for 5–10 s. The pellets should disperse, but not dissolve in the water.
8	Add 1 mL of wash buffer (labeled with 3), pre-chilled for at least 1 h at -20°C, and 5 µl wash additive (labeled with 4). Vortex until the pellet is fully dispersed. ***Note:*** *The protein pellet will not dissolve in the wash buffer.*
9	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.*
10	Centrifuge the tubes in a microcentrifuge set at maximum speed (at least 12 000 × g) for 5 min.
11	Carefully remove and discard the supernatant. A white pellet should be visible. Allow the pellet to air dry briefl y (no more than 5 min). ***Note:*** *Do not over-dry the pellet. If it becomes too dry, it will be difficult to resuspend.*
12	Resuspend the pellet in 5–40 µL of buffer I (labeled with 5). Vortex briefl y and incubate on ice for 5 min. ***Note:*** *The appropriate resuspension volume for the sample depends on a number of factors, including the protein concentration of the original sample, the capacity of the gel system used for SDS-PAGE and the sensitivity of the detection method used to visualize the proteins in the gel.* *If the pellet is large or too dry, it may be slow to resuspend fully. Sonication or treatment with the PlusOne Sample Grinding Kit can speed resuspension.*
13	Add 1 µL of buffer II (labeled with 6) for each 5 µL of buffer I used in Step 12. Vortex briefl y and incubate on ice for 5–10 min.

Step	Action
14	Add an equal volume (6–48 µL) of SDS-PAGE sample buffer (labeled with 7) to which reductant (DTT or 2-mercaptoethanol) has been added. (See preliminary preparations). If the solution turns yellowish, add buffer I in increments of 0.5 µL until the solution turns blue.
15	Vortex the sample 5–10 s and incubate at room temperature for 5–10 min. Place the sample tube in a boiling water bath or 95°C heat block for 3 min.
16	Centrifuge the tube briefl y to bring the contents to the bottom of the tube. A brief pulse is suffi cient. Gently tap the tube to ensure that the contents are mixed. The sample is now ready to be loaded onto an SDS gel.

The protein concentration of the sample is best determined using the PlusOne 2-D Quant Kit, which can accurately quantify protein in SDS-PAGE sample buffer.

Procedure B

for dilute samples of more than 100 µL

All steps should be carried out with the tubes on ice unless otherwise specified.

Step	Action
1	Transfer the protein sample into a tube that can be centrifuged at 8000 × 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 is not compatible with many plastics. This limits the choice of centrifuge tube materials.*
2	For each volume of sample, add 3 volumes of precipitant (labeled with 1). 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 (labeled with 2) to the mixture of protein and precipitant. Mix by vortexing briefl y.
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 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 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 tube. Use a pipette tip to remove the remaining supernatant. There should be no visible liquid remaining in the tubes.
7	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.
8	Add 1 mL of wash buffer (labeled with 3), pre-chilled for at least 1 h at -20°C, for each original volume of sample. Add 5 µL wash additive (labeled with 4). 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.*
9	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.*
10	Centrifuge the tubes at 8 000 × g for 10 min.
11	Carefully remove and discard the supernatant. A white pellet should be visible. Allow the pellet to air dry briefl y (no more than 5 min). ***Note:*** *Do not over-dry the pellet. If it becomes too dry, it will be difficult to resuspend.*
12	Resuspend the pellet in 5–40 µL of buffer I (labeled with 5). ***Note:*** *The appropriate resuspension volume for the sample depends on a number of factors, including the protein concentration of the original sample, the capacity of the gel system used for SDS-PAGE and the sensitivity of the detection method used to visualize the proteins in the gel. If the pellet is large or too dry, it may be slow to resuspend fully. Sonication or treatment with the PlusOne Sample Grinding Kit can speed resuspension.*
13	Add 1 µL of buffer II (labeled with 6) for each 5 µL of buffer I used in Step 12.
14	Add an equal volume (6–48 µL) of SDS-PAGE sample buffer (labeled with 7) to which reductant (DTT or 2-mercaptoethanol) has been added. (See preliminary preparations.) If the solution turns yellowish, add buffer I in increments of 0.5 µL until the solution turns blue.
15	Vortex the sample 5–10 s and incubate at room temperature for 5–10 min. Place the sample tube in a boiling water bath or 95°C heat block for 3 min.
16	Centrifuge the tube briefly to bring the contents to the bottom of the tube. A brief pulse is suffi cient. Gently tap the tube to ensure that the contents are mixed. The sample is now ready to be loaded onto an SDS gel.

The protein concentration of the sample is best determined using the PlusOne 2-D Quant Kit, which can accurately quantify protein in SDS-PAGE sample buffer.

Troubleshooting guide

Symptom: Protein pellet is difficult to resuspend in buffer I

possible cause	remedy
Pellet is too dry or a large amount of protein is present.	Use additional buffer I, sonicate or use the PlusOne Sample Grinding Kit to facilitate resuspension.

Symptom: Solution turns yellow when SDS-PAGE sample buffer is added

possible cause	remedy
Insufficient washing with wash buffer.	Add buffer I in increments of 0.5 µL until the solution turns blue.

Ordering information

Product	Quantity	Product code
SDS-PAGE Clean-Up Kit	50 samples	80648470

Related products	Quantity	Product code
Tris	500 g	17132101
Glycine	500 g	17132301
Mercaptoethanol	25 mL	17131701
Sodium Dodecylsulfate (SDS)	100 g	17131301
Urea	500 g	17131901
CHAPS	1 g	17131401
Dithiothreitol (DTT)	1 g	17131801
Bromophenol Blue	10 g	17132901
Sample Grinding Kit	50 samples, up to 100 mg tissue or cell sample	80648337
2-D Quant Kit	500 assays, 1–50 µL and up to 50 µg	80648356
2-D Clean-Up Kit	50 samples	80648451
Mini Dialysis Kit	1 kDa cut-off, up to 250 µL	80648375
Mini Dialysis Kit	1 kDa cut-off, up to 2 mL	80648394
Mini Dialysis Kit	8 kDa cut-off, up to 250 µL	80648413
Mini Dialysis Kit	8 kDa cut-off, up to 2 mL	80648432
Mammalian Protein Extraction Buffer	1 × 500 ml	28941279
Yeast Protein Extraction Buffer Kit for 10 ml cell pellet suspension	1 × kit	28944045
2-D Protein Extraction Buffer Trial Kit	for 6 × 10 ml	28943522
2-D Protein Extraction Buffer-I	for 50 ml	28943523
2-D Protein Extraction Buffer-II	for 50 ml	28943524
2-D Protein Extraction Buffer-III	for 50 ml	28943525
2-D Protein Extraction Buffer-IV	for 50 ml	28943526
2-D Protein Extraction Buffer-V	for 50 ml	28943527
2-D Protein Extraction Buffer-VI	for 50 ml	28943528
Nuclease Mix	0.5 ml	80650142
Protease Inhibitor Mix	1 ml	80650123
Vivaspin™ ultracentrifugation devices	Multiple	

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