INSTRUCTIONS

Pierce® SDS-PAGE Sample Prep Kit

89888

1728.3

Number	Description
89888	**SDS-PAGE Sample Prep Kit**, sufficient materials to prepare 50 samples for SDS-PAGE

Kit Contents:

PAGE-prep Resin, 1 ml, supplied as a DMSO-activated resin

DMSO (dimethylsulfoxide), 27 ml

PAGE-prep Elution Buffer, 5 ml

Spin Cups – Cellulose Acetate Filter, 50 each

Collection Tubes, 72 each

Lane Marker Non-reducing Sample Buffer (5X), 5 ml, contains 0.3 M Tris•HCl, 5% SDS, 50% glycerol, lane marker tracking dye; pH 6.8

Storage: Upon receipt store kit at room temperature.

Introduction

For reliable SDS-PAGE analysis, protein samples must be prepared in a buffer free of interfering substances and at protein concentrations adequate for analysis. Many interfering substances are difficult to remove by traditional sample preparation methods. The SDS-PAGE Sample Prep Kit, however, provides a simple and effective method for concentrating samples while removing many chemicals, such as acids and bases, detergents, guanidine and ammonium sulfate, that interfere with SDS-PAGE analysis (Table 1, p.3). The proprietary PAGE-prep Resin binds protein in the presence of dimethylsulfoxide (DMSO). Interfering contaminants can then be removed by washing the protein-bound resin in an easy-to-use spin cup format. Protein elution is achieved in a buffer compatible with the BCA Protein Assay, allowing quantitation of a portion of the processed sample. The eluted proteins can then be combined with sample buffer and analyzed on the SDS-PAGE system of choice.

Important Product Information

- This Sample Prep Kit is not recommended for purification or concentration of proteins in their active forms.
- Protein samples at pH 2-12 containing typical concentrations of salts (e.g., ammonium sulfate), organic solvents (e.g., acetonitrile), dyes (e.g., coomassie dye) and nonionic detergents can be effectively processed with the Sample Prep Kit.
- PAGE-Prep Resin will not effectively bind proteins in samples containing > 100 mM EDTA or > 4 M guanidine. For a list of known compatible substances, see the Substance Compatibility Table at the end of the protocol.
- Protein recovery is ~75-85% for most samples when using the recommended loading amounts (i.e., 1-70 µg of protein). After the sample has been processed, a portion of the recovered protein can be quantified using the BCA Protein Assay Kit (Product No. 23225).
- The PAGE-prep Resin cannot be regenerated and is intended for one-time use.

Additional Materials Required

- Variable-speed bench-top microcentrifuge
- Water bath or heating block set to 60°C
- Reducing agent such as 2-Mercaptoethanol (Product No. 35602), TCEP (No. 77720) or DTT (No. 20290)

Procedure for using the SDS-PAGE Sample Prep Kit

A. Resin Preparation and Sample Addition

1. Vortex the PAGE-prep Protein Binding Resin to evenly disperse the resin into a slurry.
2. Use a cut or large-orifice pipette tip to transfer 20 µl of resin slurry into the center of a spin cup.

   Note: Resin particles may clog ordinary pipette tips.
3. Based on protein concentration, add 2-300 µl of sample containing 1-70 µg total protein to the resin. Cap tube and briefly vortex.

   Note: Although 20 µl of resin may bind as much as 130 µg of protein, binding and recovery efficiency may be reduced with protein loads > 70 µg.
4. Add a volume of 100% DMSO equal to the sample volume added in Step 3. Cap tube and briefly vortex.

   Note: For best results, a minimal concentration of 50% DMSO must be maintained for optimal binding.
5. Incubate sample for 3-5 minutes at room temperature with occasional mixing to ensure maximum protein adsorption to the resin.
6. Centrifuge sample at 2,000 × *g* for 2 minutes. Discard flow-through and blot collection tube on a paper towel. Reinsert spin cup into the same collection tube.

B. Wash

1. Prepare Wash Solution by mixing 6 ml of DMSO with 6 ml of water, which makes sufficient solution for processing 20 samples. Store Wash Solution at room temperature.
2. Add 300 µl of Wash Solution to the resin. Cap tube and vortex until a homogeneous suspension is obtained
3. Centrifuge sample at 2,000 × *g* for 2 minutes. Discard flow-through and blot collection tube on a paper towel. Reinsert spin cup into the same collection tube.
4. Repeat wash one additional time for a total of two washes.

   Note: If the original sample contained large amounts of detergent, an additional wash step may be required.

C. Elution

5. Transfer the spin cup to a new collection tube and add 50 µl of Elution Buffer to the resin. Cap tube and briefly vortex to obtain a homogeneous suspension.
6. Incubate sample at 60°C for 5 minutes. After incubation briefly vortex the tube.
7. Centrifuge sample at 2,000 × *g* for 2 minutes. Discard spin cup and resin. Retain the collection tube containing eluate.

   Note: If desired perform a second elution to recover additional protein; however, this is generally not required and may result in excessive sample dilution.

   Note: Eluate may have a yellow tint; however, this will not affect downstream analysis.
8. (Optional) Assay a portion of the eluted sample for protein concentration using the BCA Protein Assay Kit.
9. Combine the appropriate sample volume for SDS-PAGE analysis with the supplied 5X sample buffer or with a sample buffer best suited for the specific electrophoresis system being used.
10. (Optional) Add reducing agent of choice (i.e., TCEP, DTT, or 2-mercaptoethanol).
11. Heat sample at 95°C for 5 minutes and proceed with SDS-PAGE according to manufacturer's recommendations.

Table 1. Concentrations of common SDS-PAGE interfering substances that are effectively removed by the SDS-PAGE Sample Prep Kit procedure.

Substance	Compatible Concentration*	Notes
Acetonitrile	66%	
Ammonium sulfate	2 M	
Dyes	N/A	Resin discoloration may occur
Strong chelating agents (EDTA)	100 mM	
Glycerol	25%	
Glycine pH 2.5	1 M	
Guanidine•HCl	4 M	
Imidazole	3 M	
Potassium thiocyanate	2 M	
Sodium chloride	1 M	
Reducing agents (DTT, TCEP, β-ME)	20 mM	Resin discoloration may occur
SDS (Detergent)	20%	
Sucrose	40%	
Triton®-X 100, Tween®-20, CHAPs, OTG (Detergents)	10%	
Tris	1 M	
Urea	6 M	

*Values represent concentrations of interfering substances that were successfully processed and do not necessarily represent the maximum.

Troubleshooting

Problem	Possible Cause	Solution
Spin cup membrane clogs during processing	High protein concentration and/or detergents	Increase the centrifugation speed to 3,000 × *g*
Poor protein recovery	Poor binding efficiency	Increase DMSO concentration to > 60% in the binding solution, increase incubation time and further dilute potentially interfering substances
	Poor elution efficiency	Perform a second elution and combine sample
		Increase elution incubation time

Related Pierce Products

25200-25244 **Precise™ Protein Gels** (see catalog or web site for a complete listing)

24590 **GelCode™ Blue Stain Reagent**, sufficient reagent to stain 20 mini gels

24612 **Pierce® Silver Stain Kit (SilverSNAP)**, sufficient reagents to stain 20 mini gels

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