INSTRUCTIONS

PIERCE
3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

PAGEprep™ Protein Clean-Up and Enrichment Kit

26800

1286.2

Number	Description
26800	**PAGEprep™ Protein Clean-Up and Enrichment Kit**, contains sufficient materials to prepare 50 samples for SDS-PAGE
	Kit Contents:
	PAGEprep™ Protein Binding Resin, 1 ml, supplied as a DMSO activated resin
	DMSO (dimethyl sulfoxide), 25 ml
	Elution/Loading Buffer, 2.5 ml supplied as a 1x stock solution; includes pink tracking dye for electrophoresis analysis
	Storage: Upon receipt store the PAGEprep™ Resin at 4°C. For long term stability store entire kit at 4°C. For daily use, store buffers at ambient temperature. Kit is shipped at ambient temperature.

Introduction

The PAGEprep™ Protein Clean-Up and Enrichment Kit contains a proprietary hydrophilic resin for the rapid preparation of protein samples for denaturing polyacrylamide gel electrophoresis (SDS-PAGE). The simple procedure involves binding of proteins to the resin in the presence of DMSO, washing and pipetting to remove soluble contaminants, and finally elution of the proteins in sample loading buffer for SDS-PAGE.

Reliable SDS-PAGE analysis requires that protein samples be dissolved in an appropriate buffer, free of interfering substances and at sample concentrations adequate for analysis. The PAGEprep™ Kit enables removal of many chemicals that interfere with SDS-PAGE analysis: guanidine, ammonium sulfate, other common salts, acids and bases, detergents, dyes, DNA, RNA, and lipids. Besides removing interfering substances, the procedure also effectively concentrates protein samples by enabling binding from a relatively dilute large-volume sample followed by elution in a smaller volume for gel loading.

Important Product Information

- The PAGEprep™ Kit is not recommended for purification or concentration of proteins in their active forms. The resin can not be regenerated and is intended for one-time use only.
- The PAGEprep™ Resin in DMSO will bind protein effectively regardless of other common sample components, such as salts (ammonium sulfate), pH 2-12; organic solvents (acetonitrile); dyes (e.g., coomassie dye) and nonionic detergents such as Triton® X-100 (Product No. 28314) and Tween®-20 (Product No. 28320). Dilute any protein solutions containing more than 4 M guanidine before use in this protocol (see Table 1 in the Additional Information section).
- PAGEprep™ Resin will not effectively bind protein in the presence of strong chelating agents such as EDTA above the concentrations specified (see Table 1 in the Additional Information section).
- PAGEprep™ Resin binds protein optimally in an organic mobile phase. A minimal concentration of 50% DMSO must be maintained for the resin to maintain binding characteristics.
- The amount of protein cannot be measured after the PAGEprep™ Procedure. The Elution/Loading Buffer is not compatible with either the BCA (Product No. 23225) or Coomassie Plus® Protein Assay (Product No. 23236). Estimate the protein content in the starting sample; then assume approximately 80% protein recovery when using the 1-20 µg loading recommended in the procedure.

Additional Materials Required

- 1.5 ml microcentrifuge tubes
- Cut or large-orifice pipette tips for delivering 20 µl of resin slurry
- Reducing agent such as 2-Mercaptoethanol (Product No. 35601), TCEP (Product No. 77720) or DTT (Product No. 20290)
- Optional: Filter sealed spin cup columns (e.g., Product No. 69700)

Material Preparation

Wash Buffer	Mix 3 ml DMSO with 3 ml H_2O to make a final concentration of 50% DMSO. This is sufficient Wash Buffer for processing 20 samples.
Elution/Loading Buffer	Equilibrate to room temperature to form a uniform solution. Mix gently.

Example Protocol for use of PAGEprep™ Protein Clean-Up and Enrichment Kit

Note: Using a filtered sealed spin cup columns (e.g., Product No. 69700) will greatly accelerate the processing time for handling multiple samples and can improve overall kit performance. Do not use frit-based spin columns (e.g., Product No. 69705) because resin may leak through the frit during processing. Visit the Pierce web site Technical Resources section for the Tech Tip describing the protocol for use of spin cup columns for this procedure.

A. Resin Preparation

1. Suspend the PAGEprep™ Protein Binding Resin by gently shaking to evenly disperse the resin into a slurry.
2. Pipette 20 µl of resin slurry into a 1.5 ml microcentrifuge tube.

   Note: Use a cut or large-orifice pipette tip to transfer the resin because of the viscosity of the slurry.

B. Sample Addition

1. Depending on protein concentration, mix 2-300 µl of protein sample containing 1-20 µg total protein with an equal volume of the supplied 100% DMSO solution to form a 50% DMSO:sample mixture.

   Note: A minimal concentration of 50% DMSO must be maintained during the binding and wash steps of the protocol.

   Note: Protein concentration cannot be measured after addition of sample to DMSO and the PAGEprep™ Resin or after elution from the resin. Estimate protein amount in the starting sample by measuring absorbance values at 280 nm or a compatible protein assay, such as the BCA (Product No. 23225) or Coomassie Plus® Protein Assay (23236).
2. Transfer 4-600 µl of the DMSO:sample mixture to the tube containing 20 µl of resin slurry. Mix the protein and slurry together for 5 seconds with a vortex mixer. Sample mixture volumes greater than 100 µl require 2-4 minutes of incubation with occasional mixing to ensure complete protein adsorption to the resin.

   Note: For optimal results, load 1-20 µg total protein onto 20 µl of resin slurry. Although in some cases this amount of resin may bind as much as 100 µg protein, the efficiency of recovery (elution) is reduced with loads >20 µg protein.
3. Centrifuge sample tube in a microcentrifuge for 2 minutes at approximately 5,000 rpm (2,000 x *g*). Collect supernatant and retain until confirmation of successful protein binding and elution is determined.

C. Wash

1. Add 0.15 ml of 50% DMSO (Wash Buffer) to the resin in the tube. Vortex briefly.
2. Centrifuge tube for 2 minutes at approximately 5,000 rpm.
3. Use a pipette to carefully remove supernatant from the resin pellet.
4. Repeat wash steps 1-3 one time.

D. Elution

1. Carefully wet and suspend the resin with 50 µl of Elution/Loading Buffer. Vortex briefly.
2. Centrifuge tube for 2 minutes at approximately 5,000 rpm.
3. Without disturbing the pellet, collect 40 µl of supernatant with a pipette and place in new microcentrifuge tube for heating.
4. Add reducing agent (i.e., 2 µl of 1 M 2-ME, 1 M TCEP or 1 M DTT) to the collected eluted sample.
5. Heat sample to 90ºC for 3-5 minutes.

   Note: After heating, samples may have a strong odor caused by trace amounts of DMSO.
6. Add eluted sample directly to wells of a polyacrylamide gel and electrophorese according to system instructions.

   Note: A pink band from the tracking dye in the Elution/Loading Buffer will act as a buffer front indicator. There is no need to add tracking dye to the heated sample.

Troubleshooting

Problem	Cause	Solution
Poor protein recovery from sample	Poor binding efficiency	Increase the DMSO concentration to above 60% in the binding solution, load more sample, increase incubation time and further dilute potentially interfering chemicals
	Poor elution efficiency	Incubate PAGEprep™ Resin/Elution Buffer mixture at 55-65°C for 5 minutes before collecting sample in steps 1 and 2 of elution

Additional Information

A. Please visit the Pierce web site for additional information on this product, including the following the Tech Tip for performing the PAGEprep procedure using Handee™ Spin Cup Columns.

B. Chemical Compatibility

Table 1. PAGEprep™ Resin Compatibility with Common SDS-PAGE Interfering Chemicals

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	
Guanadine•HCl	2 M	
Imidazole	3 M	
Potassium thiocyanate	2 M	
Reducing agents (DTT, TCEP, β-ME)	N/A	Resin discoloration may occur
Detergent (SDS)	20 %	
Sucrose	40 %	
Detergents (Triton®-X 100, Tween®-20)	10 %	
Tris	1 M	
Urea	6 M	

* Highest compatible concentration in sample before the addition of DMSO.

Related Pierce Products

69700	**Handee™ Spin Cup Columns**, 50 units
20290	**DTT**, for disulfide reduction, 5 gm
77720	**Bond-Breaker™ TCEP Solution**, for disulfide reduction, 5 ml
35600	**2-Mercaptoethanol**, for disulfide reduction, 500 gm
26681	**BlueRanger® Prestained Protein Molecular Weight Marker Mix**, 1 X 48 plate
26691	**TriChromRanger™ Prestained Protein Molecular Weight Marker Mix**, 1 X 48 plate
28378	**BupH™ Tris-Glycine-SDS Buffer Packs**, 40 packs of 25 mM Tris, 192 mM Glycine and 0.1% SDS, pH 8.3 when dissolved in 500 ml distilled water
24590	**GelCode® Blue Stain Reagent**, sufficient reagent to stain 20 mini gels
24612	**GelCode® SilverSNAP™ Silver Stain Kit II**, sufficient reagents to stain 20 mini gels
89865	**2-D Sample Prep for Soluble Proteins Kit**, for buffer exchanging soluble protein samples directly into 2-D sample loading buffer
89866	**2-D Sample Prep for Insoluble Proteins Kit**, for buffer exchanging poorly soluble protein samples directly into 2-D sample loading buffer
89863	**2-D Sample Prep for Nuclear Proteins Kit**, for extracting nuclear proteins and buffer exchanging them directly into 2-D sample loading buffer
89864	**2-D Sample Prep for Membrane Proteins Kit**, for extracting membrane proteins and buffer exchanging them directly into 2-D sample loading buffer

Product References

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Kolodziejski, P.J., *et al.* (2003). Intracellular formation of "undisruptable" dimers of inducible nitric oxide synthase. *PNAS* **100:** 14263-68.

Miller, S., *et al.* (2003). The closed structure of the MscS mechanosensitive channel: cross-linking of single cysteine mutants. *J. Biol. Chem.* **278:**32246-50.