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ISO9001

Rapid-Con™ PAGE Sample Clean-up Kit

Product Name	Qty	Cat. No.
Rapid-Con™ PAGE Sample Clean-up Kit	1 Kit	EBE-1014

Description

The Elpis Biotech Rapid-Con™ PAGE Sample Clean-up Kit enables the user to easily perform 1) rapid enrichment of diluted proteins for PAGE analysis and 2) effective removal of various contaminating substances affecting gel electrophoresis, such as guanidine, urea, detergents, nucleotides, lipids, organic solvents, or other common salts. Appropriate concentration of protein and prior removal of contaminating substances from protein samples are critical factors for obtaining highly resolved results by PAGE analysis. The Rapid-Con™ PAGE Sample Clean-up Kit contains modified silicates support which can bind proteins in an aqueous phase.
Boiling in the presence of PAGE sample loading buffer containing 1% SDS can easily liberate the bound proteins.

Kit contents

1. Protein Concentration Bead : 0.5ml of modified silicates slurry
2. 100x Bind/Wash buffer : 1 ml
3. 2x Sample Loading Buffer : 1 ml (1x = 62.5mM Tris-HCl, pH6.8, 1% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.005% BPB)

Binding capacity : <14mg/ml.
The kit contains sufficient amount of reagents for 50 preparations.
The entire kit can be stored at room temperature.
This product is guaranteed for one year from the date of purchase when handled and stored properly.

General Considerations

Protein binding compatibility with various chemicals

As shown in Table below, the Rapid-Con™ support can bind protein well in the presence of various salts, organic solvents, dyes, and detergents. However, proteins poorly bind at extreme basic condition (>pH10.0), and in the presence of SDS (>0.5%) or strong chelating agents such as EDTA (>50mM). Therefore, protein samples containing a high concentrated SDS or EDTA described below should be diluted before use in this protocol.

㈜ 엘피스바이오텍
www.elpis-biotech.co.kr
(302-854) 123-12 Chunglim-Dong, Seo-Gu, Taejeon, Korea
Tel: +82-42-581-8448. Fax: +82-42-581-8449

Table. Compatibility table for binding of Rapid-Con™ support with various chemicals.

Substances	Conc.	Notes
Guanidine HCl	>3 M	
Urea	>6 M	
NaCl, KCl	<0.5 M	<30% binding at above 0.5M
$MgCl_2$	>1 M	
Ammonium sulfate	<2 M	<50% binding at 2M
Non-ionic detergents (Triton-X 100, Tween-20)	>10%	
Reducing agents (b-ME, DTT)	>10%	
Acetonitril	>50%	
SDS	<0.5%	Remove SDS or dilute under optimal conc.
EDTA	<50 mM	Dilute below optimal conc.

Preparation of materials

1. 2x Binding buffer : 50-fold dilute of 100x washing buffer provided in the kit with distilled water.
2. 1x Washing buffer : 100-fold dilute of 100x washing buffer provided in the kit with distilled water.
3. For non-reduced condition for SDS-PAGE, user should prepare 1x gel loading buffer without reducing agent.

Protocols for use of Rapid-Con™ PAGE Sample Clean-up Kit

1. Prepare total protein (1-40μg) in protein extraction buffer (50-500 μl).
2. Add one volume of 2X Binding buffer, and 10-20 μl Rapid-Con™ protein concentration bead (protein binding capacity : <14mg/ml).
3. Suspend bead by vortexing and incubate for 10 min at room temperature.
4. Briefly centrifuge (12,000 rpm, 10-20 sec, room temperature), and discard supernatant.
5. Wash bead pellet with 300-500 μl of 1x Washing buffer by vigorous vortexing.
6. Briefly centrifuge (12,000 rpm, 10-20 sec, room temperature), and discard supernatant.
7. Repeat Steps 5-6. for complete washing.
8. Add appropriate volume of 1x gel loading buffer (10-20 μl), resuspend pellet by repeated pipetting or vortexing, and boil for 5-10 min.
9. Carefully recover eluted proteins in supernatant by brief centrifugation (12,000 rpm. 30 sec, room temperature).

Possible Troubleshooting

Poor protein recovery :

1. Dilute protein samples 2-10-fold with distilled water or low salt buffer to further dilute potentially interfering chemicals.
2. Increase incubation time.