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ISO9001

# Rapid-Con™ Protein Concentration Kit

| Product Name | Qty | Cat. No. |
|---|---|---|
| **Rapid-Con™ Protein Concentrtion Kit** | 1 Kit | EBE-1012 |

## Description

The Elpis Biotech Rapid-Con™ Protein Concentration Kit enables the user to easily perform the following routine procedures; 1) rapidly concentrate diluted proteins and 2) remove various contaminating substances in protein samples, 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 subsequent analysis such as SDS-PAGE. The Rapid-Con™ Protein Concentration Kit contains modified silicates support which can bind proteins in an aqueous phase. The bound proteins can be easily liberated by boiling in the presence of PAGE sample loading buffer, by addition of 1% SDS/5mM EDTA, or raising buffer pH up to 10.

## Kit contents

1. Protein concentration bead : 0.5 ml
2. 2x Binding buffer : 30 ml
3. Washing buffer : 60 ml
4. Elution buffer : 1 ml (50 mM Tris , pH8.0, 5 mM EDTA, and 1% SDS)

Binding capacity : <14 mg/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

General applications

1. Rapid concentration of diluted proteins extracted from tissue or cells.
2. Decontamination of interfering substances from protein samples before 1D or 2D PAGE analysis.
3. Purification of proteins from Coomassie-stained PAGE gel.
4. Concentration of soluble proteins in cell culture medium.

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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 (>50 mM). Therefore, protein samples containing a high concentrated SDS or EDTA described below should be diluted before use in this protocol.

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 0.5 M |
| $MgCl_2$ | >1 M | |
| Ammonium sulfate | <2 M | <50% binding at 2 M |
| Non-ionic detergents (Triton-X 100, Tween-20) | >10% | |
| Reducing agents (β-ME, DTT) | >10% | |
| Acetonitril | >50% | |
| SDS | <0.5% | Remove SDS or dilute below optimal conc. |
| EDTA | <50 mM | Dilute below optimal conc. |

Protein recovery

As shown in Table below, efficiency of protein recovery depends on the elution methods applied. For full elution, repeat elution step. By pooling repeated eluates, about 70-95% of total proteins can be recoverable

| Elution Methods | Temp. | Notes |
|---|---|---|
| Boiling in Laemmli sample loading buffer | 100 ℃ | >95% recovery |
| 50mM Tris-HCl (pH8.0), 5mM EDTA, and 1% SDS | RT | >70% recovery by first elution or >90% by pooling of repeated elution |
| 100mM Tris (pH9.6), 10mM EDTA | RT | <50% recovery by first elution or >70% by pooling of repeated elution |

Test was performed with BSA (5-10 μg) or tissue extracts (10-20 μg).

Preparation of materials before experiment

Suspend the support by gently shaking to evenly disperse the support into a slurry.

1.5 ml microfuge tubes.

## Protocols for use of Rapid-Con™ Protein Concentration Kit

### Protein sample enrichment and clean-up: Method I

(Concentration or decontamination of proteins in protein extraction buffer)

1. Prepare total protein (1-40 μg) in protein extraction buffer (50-500 μl).
2. Add 1 volume of 2X Binding buffer, and 10-20 μl Rapid-Con™ protein concentration bead (protein binding capacity : <14 mg/ml).
3. Suspend bead by vortexing and incubate for 10 -60 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 Wash buffer by vortexing.
6. Briefly centrifuge (12,000 rpm, 10-20 sec, room temperature), and discard supernatant.
7. Repeat Steps 5-6..
8. Add appropriate volume of elution buffer, resuspend pellet by repeated pipetting or vortexing, and then place tube for 5-10 min at room temperature.
9. Carefully recover eluted proteins in supernatant by brief centrifugation (12,000 rpm. 30 sec, room temperature).

Note: For full elution, repeat Steps 8-9. By first elution, about 70% of total protein is recoverable. By pooling eluates, about 90-95% of total proteins can be recoverable.

Note: For direct PAGE analysis of concentrated protein, 1X gel loading buffer (62.5 mM Tris, pH6.8, 1% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.005% BPB) may be used instead of elution buffer. At Step 8, add 1x gel loading buffer and boil for 2-5 min.

### Sample Concentration : Method II

(Concentration of protein in cell culture medium or in low ionic strength buffer)

1. Add 2x Binding buffer to cell culture medium to final 1x (100 μl to 500 μl).
2. Add 10-20 μl Rapid-Con™ protein concentration bead into cell culture medium and then incubate for 10 – 60 min at room temperature with mild agitation (as media volume increase, bead should be more added to efficient capturing free moving proteins to bead particles).
3. Briefly centrifuge (12,000 rpm, 10-20 sec, room temperature), and discard supernatant.
4. Wash bead pellet with 300-500 μl of Wash buffer by vortexing.
5. Brief centrifuge (12,000 rpm, 10-20 sec, room temperature), and discard supernatant.
5. Repeat Steps 3-4..
6. Add appropriate volume of elution buffer, resuspend pellet by repeated pipetting or vortexing, and then incubate for 5-10 min at room temperature.
7. Carefully recover eluted protein samples in supernatant by brief centrifugation (12,000 rpm. 30 sec, room temperature).

Note: For direct PAGE analysis of concentrated protein in cell culture medium, 1X gel loading buffer (62.5 mM Tris, pH6.8, 1% SDS, 1% β-mercaptoethanol, 10% glycerol, 0.005% BPB) may be used instead of elution buffer. At Step 6, add 1x gel loading buffer and boil for 2-5 min.

Protein elution and concentration from Coomassie-stained polyacrylamide gel

1. Stain and destain gels by conventional Brilliant blue R250 or G250 protein gel staining method. Before gel excision, rinse gels with distilled water several times.
2. Excise  protein band from stained polyacrylamide gel with razor blade, and then transfer gel slice into microfuge tube.
3. Homogenize gel slice in the presence of 5 volume of homogenizing buffer (should be prepared by user; 0.1 M sodium carbonate, pH 7.4, and 0.1% SDS).
4. Incubate gel homogenates at 37℃ for 30 min - 1 hr with occasional vortex. Optionally gel homogenates can be sonified for 5 min at room temperature to accelerate protein diffusion from polyacrylamide gel).
5. Centrifugation at 12,000 rpm for 5-10 min at room temperature.
6. Carefully recover supernatant and transfer into a fresh microfuge tube.
7. Add 1 volume of 2x Binding buffer and 10-20 ㎕ of Rapid-Con™ protein concentration bead, and suspend bead by brief vortex.
8. Place tube for 10 min at room temperature.
9. Brief centrifuge (12,000 rpm, 10-20 sec, room temperature) and discard supernatant.
10. Wash bead pellet with 300-500 ㎕ of Wash buffer by vortexing.
11. Brief centrifugation (12,000 rpm, 10-20 sec, room temperature) and discard supernatant.
12. Repeat Steps 10-11..
13. Resuspend pellet with appropriate volume of elution buffer and place at room temperature for 5-10 min.
14. Carefully recover supernatant by brief centrifugation (12,000 rpm. 30 sec, room temperature).
15. Analysis.

Note: protocol for protein elution from polyacrylamide gel is compatible with other common staining methods; metal staining, silver staining, PowerStain™ protein prestaining.

## Possible Troubleshooting

Poor protein recovery caused by poor binding :

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

Poor protein recovery caused by poor elution :

1. Repeat elution Step and pool eluates.
2. Increase concentration of SDS in elution buffer.