ProteoCon Kit

Rapid and easy protein concentration by spin column

ProteoConD kit

For:

Concentration of protein following electro elution from *denatured* polyacryamide gel (see GeBAflex-tube kit for protein extraction from PAGE).

ProteoConN kit

For:

Concentration of protein following electro elution from *native* polyacryamide gel (see GeBAflex-tube kit for protein extraction from PAGE).

From Gene Bio-Application Ltd.

April 2003

GeBAflex-tube is covered by the WO0190731 patent application assigned to Gene Bio-Application Ltd.

GeBAflex-tubes are protease, RNase, DNase and bacterial free.

All kits buffers are filtered, autoclaved and are bacterial free.

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Table of Contents

Kits Contents:

***ProteoConD* kit**	**10 units**	**20 units**
ProteoCon columns (concentration columns)	10	20
Collection tubes	10	20
ProteoConD beads	0.8 ml	1.6 ml
Buffer WB*D* (washing buffer)	15 ml	30 ml
Buffer EB*D* (elution buffer)	1 ml	2 ml
Information and protocols manual	1	1
GeBAflex-tube kit provided on request (see Ordering Information page 15)		
SDS removing buffer (provided on request)	**1 bottle**	**2 bottles**
Buffer WB*D2* (washing buffer)	30 ml	60 ml
***ProteoConN* kit**	**10 units**	**20 units**
ProteoCon columns	10	20
Collection tubes	10	20
ProteoConN beads	0.4 ml	0.8 ml
Buffer WB*N* (washing buffer)	15 ml	30 ml
Buffer EB*N*	1 ml	2 ml
Information and protocols manual	1	1
GeBAflex-tube kit provided on request (see Ordering Information page 15)		

Storage Conditions

ProteoCon kits must be stored between +4 to +30^{o}C in a dry place. Under these conditions, the Protein ***ProteoCon*** kits can be stored for up to 12 months without any deterioration in performance and quality.

Limitations on Product use

The ***ProteoCon*** kits were developed designed and are being sold for research purposes only. They are not to be used for human diagnostic purposes or drug production nor for the production of any substance intended to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the competent regulatory authorities in the country of use. All due care and attention should be exercised in the handling of materials described in this text.

Quality Control

Performances of ***ProteoCon*** kits are regularly monitored. ***ProteoCon*** kits are tested by applying it for concentration of various sizes of proteins. The quality of the concentrated protein is checked by several assays commonly used for proteins. Determination of recovery from a specific amount of protein sample provides a test of quality of the concentration achieved by ***ProteoCon*** kits.

The *ProteoCon* Kits

The technology of the ***ProteoCon*** kits is based on the bind-wash-elute protocol using a spin able column. This technology allows rapid, convenient and high performance protein concentration without any contamination.

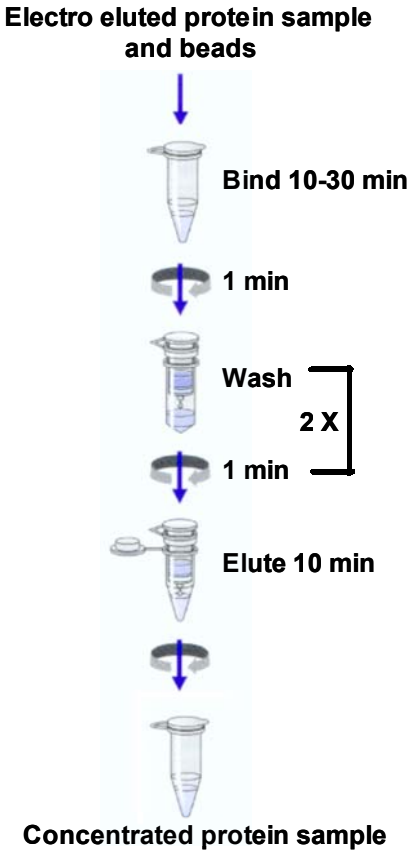
The ***ProteoCon*** kits, designed for rapid protein concentration, includes:

ProteoConD kit: use beads that are for concentration of protein which were electro eluted in denatured condition. The beads adsorb proteins at a low ionic strength phosphate buffer at neutral pH. The interaction between the charged moieties on the protein surface and the beads structure (positive and negatively charged ions centers) results in essentially a “mixed-mode” ion interaction. These are ideal for binding different types of protein and to bind small up to medium quantities of proteins. It is useful for automated protein sequencing, peptide mapping, and various biological assays.

ProteoConN kit: use beads that are for concentration of protein which were electro eluted in native condition (gel, running buffer, loading buffer and electro-elution buffer are native). The beads are strong anion exchangers, offering high capacity of

binding for proteins of all pI values. The ion exchange group is a quaternary amine, which remains charged and maintains consistently high capacities of binding over the entire working range of pH 2 to 12.

Mini Concentration Procedure



Kits Advantages

- Best method for concentration of proteins remaining active.
- Concentration procedure not requiring aggressive chemicals.
- Proteins do not aggregate during concentration protocol.
- Enables maximum recovery of proteins.
- Uses mini-spin columns.
- Easy & convenient washing of the protein from salts, detergents and other contaminations.
- Minimal loss of protein during washing.

Applications

- Concentration of protein electro-eluted from native or SDS PAGE.
- Purification of protein from gel particles or undesired salts and detergents.
- Concentration of recombinant protein after elution from nickel-column.

Yield of Molecular Recovery

Protein in denatured or native condition (BSA protein)	97%

Specifications

Maximum ***ProteoCon*** column volume capacity	700 µl
Minimum amount of protein at the start of concentration	0.5 µg
Minimum elution volume	50 µl

Tips before starting the protein concentration

1. As a rule, overestimate the amount of your sample protein.
2. If protein sample is not electro eluted or in a different buffer then those listed in the Appendix as biding buffers; and in order to guarantee binding of the protein to the beads, dialyze the protein sample with one of the recommended buffer listed in the Appendix as biding buffers.
3. Follow the suggested monitoring protocol, step by step to track the concentration progress.

ProteoConD kit

Denatured Protein Concentration

This protocol is designed to concentrate and purify proteins that have been eluted from denatured polyacrylamide gels (with GeBAflex-tube kit, see Ordering Information, page 15) using running buffer containing SDS or other denatured compounds. This protocol also purifies proteins from SDS, urea, and other salt residues.

IMPORTANT: ***ProteoConD*** kit was tested to concentrate proteins that were electro eluted using denatured 1X PRB, MOPS or MES running buffer (see buffers composition on page 12). If a different running buffer is used, follow the monitoring steps in the course of the concentration protocol to ensure that the binding, washing and elution are occurring.

Concentration Protocol

1. **Resuspend *ProteoConD* beads by vortexing for 1 min. Transfer the desired amount of beads from the *ProteoConD* tube to a fresh 1.5 ml microcentrifuge tube. For 1 µg of protein take 3 µl of *ProteoConD* beads.**

   For example, take 30 µl of ***ProteoConD*** beads for 10 µg of protein sample.

2. **Equilibrate the *ProteoConD* beads by adding 500 µl of buffer WB*D*; mix the tube vigorously by vortex for 10 seconds.**

3. **Pellet the beads by centrifuge the tube at +4°C for 1 min at 14,000 RPM. Remove the solution without disturbing the *ProteoConD* beads pellet.**

4. **To bind the protein, apply the solution containing the protein sample to the equilibrated *ProteoConD* beads obtained in Step 3.**

5. **Shake the tube containing the protein sample from Step 4 for 30 min at +4°C. To insure maximum binding, mix gently by inverting the tube 4 times every 5-10 min during the incubation.**

To keep protein activity for downstream protocols, it is recommended to perform all steps of the concentration protocol at +4°C.

6. **Place a *ProteoCon* column in the provided 2 ml collection tube.**

7. **Apply the sample from Step 5 to the *ProteoCon* column, and centrifuge at +4°C for 1 min at 5,000 RPM.**

   The maximum volume of the concentration column is 700 µl. For sample volumes of more than 700 µl, simply load and spin again.

8. **Discard flow-through and place the *ProteoCon* column in the same collection tube.**

   Collection tubes are re-used to reduce plastic waste.

   Binding monitoring: TCA-precipitate the flow-through and save it for an analytical gel (sample 1) to determine whether any unbound protein was remained in the flow-through (TCA precipitation protocol - see page 9).

9. **To wash, add 0.5 ml of WB*D* buffer to the *ProteoCon* column and mix by tapping on the tube.**

10. **Centrifuge at +4°C for 1 min at 5,000 RPM.**

11. **Discard flow-through and place the *ProteoCon* column in the same collecting tube.**

    Wash monitoring: TCA-precipitate the flow-through and save for an analytical gel (sample 2) to determine whether the protein was release by the washing buffer (TCA precipitation protocol, see page 9).

12. **Repeat Steps 9-11 once more.**

    Important: If SDS needs to be removed from the protein sample, use WB*D2* (provided on request from Gene Bio-Application Ltd, Cat number PDS010, see page xx) instead of WB*D* buffer and repeats Steps 9-11 at least 5 times.

13. **Insert the *ProteoCon* column in a clean 1.5 ml microcentrifuge tube.**

14. **To elute the protein, add 50-100 µl of EB*D* Buffer directly to the *ProteoConD* beads, gently pipette up and down the beads with a 200 µl tip, incubate for 10 min at +4°C. Centrifuge at +4°C for 1 min at 5,000 RPM.**

Important: If a precipitate is appearing in the EB*D* Buffer tube, incubate the tube at 37°C till the precipitate is disappearing.

Watch carefully not to damage the filter inside the concentration column tube during the pipetting up and down. The pipetting up and down action will provide better mixing of the elution buffer with the beads. Make sure that the elution buffer is dispensed directly onto the ***ProteoConD*** beads for complete elution of the bound protein.

Elution monitoring: Re-elute the ***ProteoConD*** beads with 50 µl 2X Protein Loading Buffer (2XPLB) and save for an analytical gel (sample 3) in order to determine whether the protein was not released from the beads by the buffer EB*D* (for recommended elution buffer 2XPLB, see page 12).

Important: Store the concentrated protein under appropriate conditions. If needed, dialyze the sample using the *GeBAflex-tube* kit from Gene Bio-Application Ltd (see Ordering Information page 14).

ProteoConN kit

Native Protein Concentration

This protocol is designed to concentrate and purify proteins that have been eluted from **native** polyacrylamide gels (no SDS or other denatured compounds, see ***GeBAflex-tube*** kit for electro elution protocol). This protocol also serves to purify proteins from urea and other salt residues.

IMPORTANT: ***ProteoConN*** kit was tested for concentration of proteins, which were electro eluted, using native 1X PRB, MOPS or MES running buffers (see buffers composition, page 12). If a different buffer is used, follow the monitoring steps in the course of the concentration protocol to ensure that the binding, washing and elution are occurring.

Concentration protocol

1. **Add the desired amount of *ProteoConN* beads to a 1.5 ml microcentrifuge tube containing the protein sample. For 1 µg of protein add 1.5 µl of *ProteoConN* beads.**

   For example, add 15 µl of ***ProteoConN*** beads for 10 µg of protein sample.

2. **To bind the protein, shake the tube containing the protein sample for 10 min at +4°C. To insure maximum binding, mix gently by inverting the tube 4 times every 2 min during the incubation.**

   To keep protein activity for down-stream protocols, it is recommended to run all the concentration protocol at +4°C.

3. **Place the *ProteoCon* column in a provided 2 ml collection tube.**

4. **Apply the solution from Step 2 to the *ProteoCon* column.**

   The maximum volume of the ***ProteoCon*** column is 700 µl. For sample volumes exceeding 700 µl, simply load and spin again.

5. **Spin the sample at +4°C for 1 min at 5,000 RPM.**

6. **Discard flow-through and place the *ProteoCon* column in the same collection tube.**

   Collection tubes are re-used to reduce plastic waste.

   Binding monitoring: TCA-precipitate the flow-through and save for an analytical gel (sample 1) to determine whether any unbound protein has remained in the flow-through (for TCA precipitation protocol, see page 9).

7. **To wash, add 0.5 ml of WB*N* Buffer to the *ProteoCon* column and mix by tapping on the tube.**

8. **Centrifuge at +4°C for 1 min at 5,000 RPM.**

9. **Discard flow-through and place the *ProteoCon* column in the same collecting tube.**

   Washing monitoring: TCA-precipitate the flow-through and save for an analytical gel (sample 2) in order to determine whether the protein was released by the washing buffer (for TCA precipitation protocol, see page 9).

10. **Repeat Steps 7-9 once more.**

11. **To elute the protein, insert the *ProteoConN* column in a clean 1.5 ml microcentrfuge tube.**

12. **Add 50-100 µl Buffer EB*N* on the *ProteoConN* beads, gently pipette up and down with a 200 µl tip, incubate for 10 min at +4°C.**

    Important: Watch carefully not to damage the filter inside the concentration column tube during the pipetting up and down. The pipetting up and down will mix the elution buffer with the beads. Ensure that the elution buffer is dispensed directly onto the ***ProteoConN*** beads for complete elution of the bound protein.

    Elution monitoring: Re-elute the ***ProteoConN*** beads with 50 µl 2X Protein Loading Buffer (2XPLB) and save for an analytical gel (sample 3) in order to determine whether the protein was not released from the beads by the buffer EL*N* (for recommended 2XPLB, see page 12).

13. **Centrifuge at +4°C for 1 min at 5,000 RPM.**

    Store the concentrated protein under appropriate conditions. If needed, dialyze the sample using the *GeBAflex-tube* kit from Gene Bio-Applications Ltd (see Ordering Information page 15).

Trichloroacetic acid (TCA) precipitation procedure

1. Add equal volume of 20% TCA to the microcentrifuge tube containing the protein solution and mix thoroughly.

   For example, add 700 µl of 20% TCA to a 700 µl sample.

2. Incubate for 60 min on ice.

3. Spin in a microcentrifuge at +4°C for 30 min at 14,000 RPM.

4. Discard the supernatant carefully.

5. Add 500 µl cold acetone.

6. Incubate at -20°C for 30 min and centrifuge the sample at +4°C for 30 min at 14,000 RPM.

   To increase protein precipitation yield, incubate the samples overnight at –20°C.

7. Discard the supernatant and air-dry the pellet.

8. Resuspend the pellet using 0.1M NaOH or dH_2O (**use at least 20 µl to perform resuspension**). If dH_2O is used for resuspension, incubate the sample for 5 min in 60°C, resuspend the sample and incubate 5 min more at 60°C.

Troubleshooting Guide

Important: Following the suggested monitoring protocol of the binding, washing and the elution steps is the best way to analyze problems occurring during concentration protocol.

Problem	Cause	Comments and Suggestion
Low recovery yields at the end of the concentration protocol.	Insufficient mixing in the binding step	Mix thoroughly by inverting the tube.
	Not enough *ProteoCon* beads applied at the binding step	Add more *ProteoCon* beads.

	Pipetting did not involve the *ProteoCon* beads at the elution step	Do pipetting on the *ProteoCon* beads at the elution step.
	Insufficient incubation time at the binding step	Prolong binding time at the binding step.
	Insufficient incubation time at the elution step	Prolong elution time at the elution step.
	Use of unsuitable type of *ProteoCon* beads at the binding step.	For denatured binding buffer use *ProteoConD* kit. For native binding buffer use *ProteoConN* kit.
ProteoCon beads found in the collecting tube after washing step.	Damaged concentration column tube	Change to a new concentration tube.
ProteoCon beads found in the collecting tube after elution step.	Concentration column tube filter damaged by the 200 µl tip	Do gentle pipetting on the wall of the concentration column tube onto the *ProteoCon* beads.
ProteoCon beads get stuck when pippetted from the *ProteoCon* beads stock solution tube.	No mixing of the *ProteoCon* beads stock solution tube.	Mix vigorously by vortex the *ProteoCon* beads stock solution tube.
	Head of the 200 µl tip left uncut.	Cut 2 mm off the head of the 200 µl tip to facilitate pipetting up the ProteoCon beads.

Ordering Information

Product	**Contents**	**Cat. No.**

ProteoConN kit + GeBAflex-tube	10 concentration columns, beads, native buffers. 10 GeBAflex-tubes, 3500 MWCO, supporting tray and 2 handbooks.	PNT010
ProteoConN kit + GeBAflex-tube	20 concentration columns, beads, native buffers. 20 GeBAflex-tubes, 3500 MWCO, supporting tray and 2 handbooks.	PNT020
ProteoConD kit + GeBAflex-tube	10 concentration columns, beads, denatured buffers. 10 GeBAflex-tubes, 3500 MWCO, supporting tray and 2 handbooks.	PDT010
ProteoConD kit + GeBAflex-tube	20 concentration columns, beads, denatured buffers. 20 GeBAflex-tubes, 3500 MWCO, supporting tray and 2 handbooks.	PDT020
SDS removing buffer	15 ml SDS removing buffer (WB*D*2)	PDS010
SDS removing buffer	30 ml SDS removing buffer (WB*D*2)	PDS020
Related Products		
GeBAflex-tube (30)	30 *GeBAflex-tubes* of 3500 cut-off, buffers, supporting tray, floating rack	GeBA-T012
GeBAflex-tube (30)	30 *GeBAflex-tubes* of 6000-8000 cut-off, buffers, supporting tray, floating rack	GeBA-T022
SeeBand protein staining solution (500 ml)	500 ml of **non-fixative** protein staining solution	SB010
SeeBand *Forte* protein staining solution (500 ml)	500 ml of fixative protein staining solution	SB020

Appendix

Composition of denatured buffers for *ProteoConD* kit:

Electro elution buffers (binding buffers)

1. **PRB X1: 0.192M Glycine, 0.025M Tris-Base and 0.1% SDS.**
2. **MOPS X1: 50mM MOPS, 50mM Bis-Tris, 0.1% SDS and 1mM EDTA.**

3. **MES X1: 50mM MES, 50mM Bis-Tris, 0.1% SDS and 1mM EDTA.**

Washing buffers:

4. **Buffer WB*D*: 0.01M Sodium Phosphate (monobasic) pH 6.8.**
5. **Buffer WB*D2*: provided on request by Gene Bio-Application Ltd, Cat # PDS010 for removing SDS from protein (see Ordering Information).**

Elution buffer:

6. **Buffer EB*D*: 0.8 M Sodium Phosphate (mono-basic) pH 6.8 and 0.1 % n-Dodecyl β-$_D$-Maltoside.**

Composition of native buffers for *ProteoConN* kit:

Electro elution buffers (binding buffers)

7. **PRB X1: 0.192M Glycine and 0.025M Tris-Base.**
8. **MOPS X1: 50mM MOPS, 50mM Bis-Tris, and 1mM EDTA.**
9. **MES X1: 50mM MES, 50mM Bis-Tris, and 1mM EDTA.**

Washing buffer:

10. **Buffer WB*N*: 0.020M Tris-Hcl (pH 9).**

Elution buffer:

11. **Buffer EB*N*: 0.5 M Sodium Chloride and 0.0125% Triton-X100.**

Beads re-elution buffer

12. **2XPLB: 4% SDS, 100mM Tris-Hcl pH 6.8, 0.2% Bromophenol blue, 2% Glycerol and 200 mM DTT.**