G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Column-PROTEIN-Concentrate™

Concentration of Dilute Protein Solutions

(Cat. # 786-126)

INTRODUCTION

Many precipitation techniques are currently used for concentrating dilute protein solutions. Some of the precipitation techniques are not suitable for concentrating large volumes of dilute protein solutions. Furthermore, many fragile and enzymatic proteins may lose their biological activity when concentrated by acid precipitation techniques. Conventional chromatography techniques are only useful for concentrating dilute protein solution when the binding & elution characteristics of the protein are established. Yet another limitation of the conventional chromatography is that they require large elution volumes. The Column-PROTEIN-Concentrate™ Kit has been specifically developed for concentration of those proteins that cannot be concentrated either by precipitation or other techniques. The kit is based on a proprietary Protein Binding Resin that binds and immobilizes any protein in low salt buffer between pH 2-12 (capacity ~ 0.5mg protein/ml Protein Binding Resin). The immobilized protein is spin-eluted in a small volume of specifically formulated elution buffer, giving several fold effective concentration. This kit is suitable for concentration of a total of 4mg protein in either single or multiple procedures.

ITEM(S) SUPPLIED (Cat. # 786-126)

Description	Size
Protein Binding Resin	10ml
Protein Elution Buffer [2X]	30ml
Spin Columns, 3ml	5
SpinOUT™ GT-600, 3ml	5
Collection Tubes	5
Caps	5

STORAGE CONDITION:

The kit is shipped at ambient temperature. Upon arrival, store at 4°C.

ADDITIONAL ITEMS REQUIRED

Centrifuge tubes and centrifuge.

IMPROTANT INFORMATION

1. To help protect your protein, perform the entire procedure in a cold room or on ice.
2. The binding capacity of the Protein Binding Resin is approximately 0.4mg protein per 1ml packed resin.

PREPARATION BEFORE USE

Protein Binding Buffer

1. The optimal Protein Binding Buffer should be the same composition as the buffer the protein sample is solubilized in, however the total salt concentration must be <20mM.
   An alternative Protein Binding Buffer is 50mM Tris, 5-10mM NaCl, pH 6.6-8.5.

Sample Preparation

Due to the binding properties on the Protein Binding Resin, the protein sample must be in a buffer containing<20mM salt.

- If the buffer has no salt; add 5µl Protein Elution Buffer [2X] to every 1ml protein solution to increase the salt concentration to 10mM.
- If the buffer has >20mM salt, dilute the protein solution with ultra pure water to achieve a final salt concentration of ~10mM.
- Alternatively, dialyze the protein solution against the Protein Binding Buffer for 4-5 hours.
  NOTE: *For the best result, the protein-buffer and the buffer used for preparation and equilibration of the column must be identical.*

PROTOCOL

Protein-Column-Concentrate™ Preparation

1. Gently shake the bottle of Protein Binding Resin to obtain an homogenous slurry. Transfer an appropriate volume of 50% slurry to a column. 1ml slurry is equivalent to 0.5ml resin.
2. Allow the storage buffer to flow through under gravity.
3. To equilibrate the column, apply 10 column volumes (CV) of Protein Binding Buffer to the column and allow to flow through.

Protein Concentration

1. Apply 2-3ml aliquots of protein solution to the top of the column. Allow the protein solution to pass through the column and collect the eluent in the collection tube. Transfer to a suitable container and save until the concentrated protein has been analyzed. Repeat until the entire protein solution has been applied to the column.
2. Reposition the column in the collection tube and centrifuge the column for 30 seconds at 200xg. Combine flow through with the above flow through
   NOTE*: Centrifugation should not be too severe to dry the column. Centrifugation should be at such a moderate speed that it removes only 60-70% of the buffer from the column, leaving behind in the column 30-40% buffer. If necessary, make a trial run (before loading the protein sample) to determine an appropriate centrifugation condition.*
3. Reposition the column into the collection tube.
4. Prepare 1X Protein Elution Buffer by mixing equal volumes of 2X Protein Elution Buffer and Protein Binding Buffer.
5. For each column volume, apply 0.25 CV 1X Protein Elution Buffer. For every 1ml resin, add 0.25ml 1X Protein Elution Buffer.
6. Incubate the column for 5 minutes.
7. Centrifuge the column as before (Step 2).
8. Re-apply the eluent to the top of the column and incubate for a further 2 minutes. Centrifuge the column as before (Step 2).
9. Re-apply the eluent as before and incubate for a further 2 minutes. Centrifuge the column at twice the speed and time as the previous centrifugations to collect the concentrated protein solution in the collection tube.
   NOTE*: A small percentage (5-10%) of protein may still remain in the column. Any remaining protein may be recovered by eluting with 0.1ml 1X Protein Elution Buffer for every 1ml resin bed and repeating steps the procedure steps 6-9.*
10. Exchange the buffer of the eluted protein solution with the buffer of your choice. See Appendix for performing a buffer exchange with the supplied SpinOUT™ GT-600.

COLUMN REGENERATION

1. Column may be regenerated and used one more time.
2. For regeneration, apply 1ml 2X Protein Elution Buffer for each 1ml resin bed.
3. Incubate for 5 minutes.
4. Wash the column with 20 volumes of pure water.
5. Store the column at 4°C in 30% ethanol.
6. Follow Protein-Column-Concentrate™ preparation as before.
   NOTE: *Additional elution buffer may be purchased separately.*

APPENDIX: SpinOUT™ PROTOCOL

Preparation Before Use

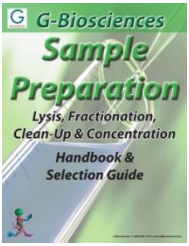
1. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.
2. Prepare the SpinOUT™ column by centrifuging the SpinOUT™ columns at 1,000g for 1 minute to compact the resin.
3. Remove the top and then bottom caps. Place into an appropriate collection tube.
4. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.

Protocol: Buffer Exchange

1. Place the column in a new collection tube and remove the cap.
2. Add 1.5ml buffer to be exchanged into to the column
3. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
4. Repeat steps 2 and 3, a further five more times, ensuring the buffer is discarded after each centrifugation.
5. Place the column in a new collection tube and remove the cap.
6. Slowly, apply 0.3-0.9ml protein solution to the center of the SpinOUT™ resin. ***NOTE:*** *The recommended load volume is a guideline. The actual volumes used will be dependent on your sample, the concentration of salts and contaminants to be removed and the recovered purity desired. For optimal removal of contaminants, we recommend using a sample volume of <20% of the resin bed volume. Loading more than the recommended load volume will result in a higher level of contaminating salts and other molecules.*
7. Centrifuge the column at 1,000g for 6 minutes to collect the protein solution. Discard the column.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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Last saved: 6/17/2015 TN

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