Column PROTEIN Concentrate™ Kit

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.

APPLICATIONS: Concentration of dilute protein solution.

ITEMS SUPPLIED: **Cat # 786-126**

Protein Binding Resin	10ml
Protein Elution Buffer [2X]	30ml
Spin Columns, empty	5
SpinOUT, GT-600 (Medi)	5
Collection Tubes	5

STORAGE CONDITION:

Shipped at ambient temperature. Upon arrival, store at 4°C.

ADDITIONAL ITEMS NEEDED: Centrifuge tubes and centrifuge.

PROTOCOLS

Perform the entire procedure in a cold room, unless the protein can survive concentration at room temperature. The Protein Binding Resin binds and immobilizes approximately 0.4mg protein per 1ml packed resin. Prepare an appropriate size column using the Protein Binding Resin, as follows.

1. Prepare Protein-Buffer for Protein Binding & Concentration:

The column for concentrating protein solution should be prepared and equilibrated with the protein-buffer, i.e., the same buffer in which the protein solution is prepared, which contains less than <20mM salt concentration (e.g., sodium, potassium, calcium salts, etc.).

Check the composition of your protein-buffer. If the buffer does not contain salt add [2X] Elution Buffer (5µl/ml buffer) to increase the ionic strength to 10mM. If the protein-buffer contains salt concentration higher than 20mM, dilute the protein-buffer with pure water to reduce the salt concentration to around 10mM.

Alternatively, prepare a new protein-buffer containing all of the components of your protein-buffer, except reduce the salt concentration to around 10mM. The composition of one such buffer suitable for concentrating protein solution is: **1-50mM Tris, 5-10mM NaCl, pH 6.6-8.5.**

2. Prepare A Protein Concentration Column: The kit is supplied with 5 empty columns. Each column has the capacity to hold up to 3ml resin. Each 1ml volume position is marked with a ring around the column. Additional columns may be purchased separately. Position a column in a tube or a test tube rack.

Shake the bottle containing Protein Binding Resin to fully suspend the resin. Transfer an appropriate volume of the fully suspended resin into a column (each 1ml fully suspended column will pack approximately into ½ ml resin-bed.). Allow the column to drip and the resin to pack into the column tightly.

3. Equilibrate the Protein Concentration Column: Pass 10-15 volumes of the protein-buffer through the column (i.e., for each 1ml packed resin pass 10-15ml protein-buffer). Apply an aliquot of 2-4ml of protein-buffer on top of the column and allow the buffer to drip into a collection tube. After a final aliquot of protein-buffer, allow the column to drip until there is no buffer dripping from the column.

4. Prepare The Protein Solution For Concentration:
For the best result, the protein-buffer and the buffer used for preparation and equilibration of the column must be identical. Check the composition of protein-buffer. If the buffer does not contain salt add [2X] Elution Buffer (5µl/ml buffer) to increase the ionic strength of the buffer to 10mM. If the buffer's salt concentration is higher than 20mM (e.g., sodium, potassium, calcium salts, etc.), dilute the protein solution with pure water to reduce the salt concentration to around 10mM. Alternatively, Dialyze the protein solution in low salt buffer prepared in step1 for 4-5 hours.

5. Apply The Protein Solution For Concentration:
Read the ***NOTE*** below first.
After the column has been equilibrated, apply the protein solution on top of the column in small aliquots of 2-3ml each. Allow the protein solution to pass through the column and collect the eluent in a clean tube. When the entire protein solution has been loaded on the column, allow the column to drip until there is no buffer coming out of the column.

6. Empty the collection tube and save the column eluent until a satisfactory result is concluded.
Reposition the column into the collection tube and centrifuge the column for 30 seconds at 200xg. Make note of the centrifugation speed and the length of centrifugation.

> ***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.*

7. ELUTION: Empty the collection tube and reposition the column into the collection tube. Prepare [1X] elution buffer by mixing equal volume of [2X] Elution Buffer and protein-buffer. For each 1ml resin bed apply 0.25ml [1X] elution buffer. Allow the buffer to pass through the column and collect any eluent in the collection tube. Incubate the column for 5 minutes.

8. Centrifuge the column for 30 seconds at 200xg, or at exactly the same centrifugation condition used in step 6 (i.e., the same speed and time).

9. Re-apply the eluent collected in the collection tube on top of the column and incubate for 2 minutes. Centrifuge again at exactly the same centrifugation condition used in step 6.

10. Repeat step 9 one more time. Centrifuge at double the speed and the length of time. Collect the 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 elution with 0.1ml elution buffer/1ml resin bed and repeat the procedure steps 7-10.*

11. Exchange the buffer of the eluted protein solution with the buffer of your choice. Pass the eluted protein solution through a pre-equilibrated SpinOUT- GT-600 column. (Follow the instructions for SpinOUT columns).

> ***Related Note****: Column may be regenerated and used one more time. For regeneration, apply [2X] elution buffer, 1ml elution buffer for each 1ml resin bed. Incubate for 5 minutes. Wash the column with 20 volumes of pure water. Store the column in cold in 30% ethanol-water. Equilibrate the column as in step 3 before use. Additional elution buffer may be purchased separately.*

Related Products

For related products, please visit our web site www.GBiosciences.com or contact us.