Aurum™ Ion Exchange Mini Kits and Columns

Instruction Manual

Catalog #	
732-6710	Aurum AEX Mini Kits, 2 pk
732-6705	Aurum AEX Mini Kits, 10 pk
732-6706	Aurum AEX Mini Columns, 25 pk
732-6707	Aurum AEX Mini Columns, 100 pk
732-6711	Aurum CEX Mini Kits, 2 pk
732-6702	Aurum CEX Mini Kits, 10 pk
732-6703	Aurum CEX Mini Columns, 25 pk
732-6704	Aurum CEX Mini Columns, 100 pk

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Section 1
Introduction

Proteomics Sample Fractionation

Sample preparation plays an ever-increasing role in proteomics research. Selective separation techniques prior to multidimensional analysis, such as 2-D gel electrophoresis, HPLC-MS, or HPLC-CE, can significantly improve the chances of resolving complex protein mixtures into their individual proteins. Among these techniques are anion exchange (AEX) and cation exchange (CEX) chromatography. Aurum AEX and CEX columns allow rapid fractionation of complex protein mixtures in only a few steps using the common technique of ion exchange chromatography.

Ion Exchange Chromatography

Ion exchange chromatography can be used to concentrate and purify proteins based on their ionic charge or isoelectric point (pI) at a given pH. At the isoelectric point of a protein, its net charge is zero. At a pH higher than the pI of the protein, the protein will be negatively charged and will bind to an AEX resin. At a pH lower than the pI, the protein will be positively charged and will bind to a CEX resin. When an ion is applied to an ion exchanger of opposite charge, it is adsorbed to the resin, while neutral ions or ions of the same charge are eluted in the flow-through fraction. Binding of the ions is reversible, and adsorbed molecules are commonly eluted with salt or a change of pH.

Table 1. Choosing the appropriate column.

Resin Type	Protein of Interest
AEX	Acidic
CEX	Basic

The choice of whether to use an anion or cation exchanger is determined mainly by the pI, and the relationship between pH and the activity or stability of the protein of interest. Once the type of ion exchanger is determined, the choice of buffer and pH is also determined by the relationship between pH and activity.

Resins and Buffers Description

Each Aurum AEX and CEX column contains 0.2 ml of UNOsphere™ Q or S support, unique media based on a proprietary polymerization process. Both the UNOsphere Q and S ion exchange media were developed to deliver efficient protein capture and high binding capacity. The Aurum AEX and CEX

columns have binding and elution buffers included to afford minimal handling and ease of use. The formulations for each of the buffers are outlined in Table 2.

Table 2. Properties of Bio-Rad ion exchange resins.

	Aurum CEX	Aurum AEX
Resin type	UNOsphere S cation support	UNOsphere Q anion support
Binding capacity	12 mg IgG*/column	36 mg BSA*/column
Column bed volume	0.2 ml	0.2 ml
Binding buffer	20 mM sodium acetate, pH 5.0	20 mM Tris, pH 8.3
Elution buffer	Binding buffer + 1.0 M NaCl	Binding buffer+ 1.0 M NaCl

*Binding capacity determined using IgG (UNOsphere S) and BSA (UNOsphere Q). Capacity will differ between proteins and will depend on the exact loading conditions.

A broad range of buffer systems can be used with Aurum AEX and CEX columns, depending on the protein of interest or specific process required. The chemical stability and broad operating pH range of the UNOsphere ion exchange media allow the use of a variety of buffers. Buffers commonly used for AEX or CEX can be used in place of the provided buffers (see Table 3).

The best results are achieved when buffering ions that have the same charge as the functional group on the ion exchanger are used, e.g., phosphate paired with a cation exchanger, or Tris paired with an anion exchanger. As a general rule, the pH of the experiment should fall within the buffering range of the chosen buffer (see Table 3). This permits use of the lowest possible buffer concentration while maintaining maximum buffering capacity. In most cases, a buffer concentration of 20 mM is recommended.

Table 3. Common buffers for ion exchange chromatography.

Cation	Buffering Range	Anion	Buffering Range
Acetic acid	4.8–5.2	Bicine	7.6–9.0
Citric acid	4.2–5.2	Bis-Tris	5.8–7.2
HEPES	7.6–8.2	Diethanolamine	8.4–8.8
MES	5.5–6.7	Diethylamine	9.5–11.5
MOPSO	6.5–7.9	L-histidine	5.5–6.0
Phosphate	6.7–7.6	Imidazole	6.6–7.1
PIPES	6.1–7.5	Pyridine	4.9–5.6
TES	7.2–7.8	Tricine	7.4–8.8
Tricine	7.8–8.9	Triethanolamine	7.3–8.0
		Tris	7.5–8.0

Sample Preparation

Proper adjustment of the sample pH and ionic strength is critical for consistent and reproducible chromatography. For best results, the sample should be exchanged into the loading buffer or diluted to the buffer's concentration. Buffer exchange can be accomplished using Micro Bio-Spin™ 6 (catalog #732-6221) or Bio-Spin® 6 (catalog #732-6227) columns, Econo-Pac® Bio-Gel® P-6 cartridges (catalog #732-0011), or Econo-Pac 10DG desalting columns (catalog #732-2010). The correct product to use will be determined by the volume of the sample. Always centrifuge or filter the sample (0.2–0.45 µm filter) to remove particulates.

Use of Aurum Ion Exchange Columns to Concentrate Protein Solutions

The Aurum AEX and CEX columns are not only purification tools but also can be used to concentrate target proteins of interest in dilute solutions, a critical factor for subsequent analyses such as 2-D electrophoresis. Protein solutions of 0.1–0.2 mg/ml can be concentrated 30–70-fold using the appropriate resin with little loss of protein.

Table 4. Enrichment of typical proteins with Aurum AEX and CEX columns.

		Initial Solution		Final Solution	
Resin	**Protein**	**Volume**	**Concentration**	**Enrichment**	**Recovery**
AEX	BSA	30 ml	0.1 mg/ml	70–fold	90%
CEX	Cytochrome c	30 ml	0.2 mg/ml	60–fold	95%

Bovine serum albumin (BSA) and cytochrome c were dissolved in buffer* and 30 ml loaded on an Aurum AEX or CEX column, respectively, in 1 ml aliquots. No protein was detected in the unbound fractions. The columns were eluted with 2 x 300 µl washes* containing 1 M NaCl. Protein concentrations were determined using the *DC*™ protein assay.

*BSA dissolved and washed in 20 mM Tris, pH 8.3; cytochrome c dissolved and washed in 20 mM sodium acetate, pH 5.0

Ability to Purify Proteins of Differing pI

Three proteins, ovalbumin (MW 45,000, pI 4.6), conalbumin (MW 77,000, pI 6.9), and cytochrome c (MW 12,000, pI 10.7), were completely separated using Aurum CEX and AEX columns in tandem. The respective unbound and bound fractions were analyzed by 4–20% SDS-PAGE (Figure 1). The three proteins were equilibrated in 20 mM sodium acetate buffer, pH 5.0, and applied to the CEX column. At this pH, the ovalbumin (pI 4.6) passed through the column while the conalbumin (pI 6.9) and cytochrome c (pI 10.7) remained bound to the column. The bound fractions were then eluted with elution buffer, desalted, buffer exchanged into 20 mM Tris, pH 8.3, and then applied to the AEX column at pH 8.3. The conalbumin remained bound while the cytochrome c was recovered in the unbound fraction. All three proteins were recovered with an estimated purity of >95% and a total recovery of >90%.

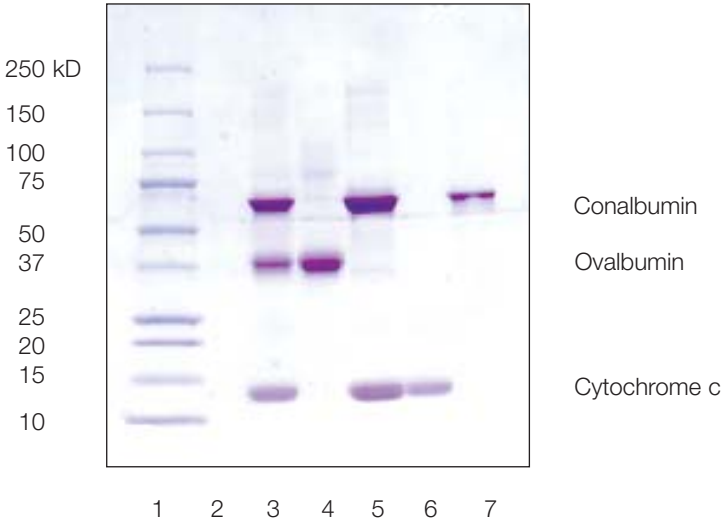


Fig. 1. Separation of three test proteins on Aurum AEX and CEX columns.

The Aurum CEX column was equilibrated in 20 mM sodium acetate buffer, pH 5.0. The mixture of three proteins (lane 3, 0.4 ml at 30 mg/ml total protein) was added to the column. The unbound protein containing ovalbumin was collected (lane 4). The bound fraction containing conalbumin and cytochrome c (lane 5) was eluted with 2 x 300 µl washes with 20 mM sodium acetate buffer, pH 5.0, containing 1 M NaCl. The eluates from the washes were desalted and buffer exchanged using Micro Bio-Spin 6 desalting columns (catalog #732-6221) and applied to an Aurum AEX column. The unbound fraction containing cytochrome c was collected (lane 6), while the bound fraction containing conalbumin (lane 7) was recovered with 2 x 300 µl washes of 20 mM Tris, pH 8.3, containing 1 M NaCl. The fractions were assayed for protein using the Bio-Rad protein assay to determine protein recovery. Purity was estimated from SDS-PAGE analysis on a 4–20% linear gradient Criterion™ Tris-HCl gel (catalog #345-0033). The gel was stained with Coomassie Blue. Bio-Rad Precision Plus Protein™ standards (catalog #161-0373) are shown in lane 1.

Section 2
Components

The Aurum ion exchange products contain the following components:

	2 pk	10 pk	25 pk	100 pk
Aurum AEX or CEX columns	2	10	25	100
Collection tubes, 2.0 ml	15	–*	–*	–*
Plastic test tubes, 12 x 75 mm	2	10	–†	–†
AEX or CEX binding buffer	15 ml	50 ml	–	–
AEX or CEX elution buffer	15 ml	50 ml	–	–
Micro Bio-Spin 6 columns, Tris buffer	4	20	–	–
Instruction manual	1	1	1	1
Protocol overview	1	1	1	1

*Order catalog #223-9430, EZ Micro™ test tubes, 2 ml, 500

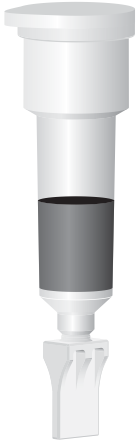
†Order catalog #732-6714, Test tubes, 12 x 75 mm, 100/bag

Section 3
Storage Conditions

Solutions and columns should be stored at 4°C. Do not freeze. Shelf life is 12 months at 4°C.

Section 4
Necessary Supplies

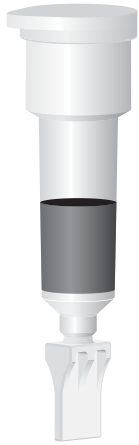
- Microcentrifuge (≥10,000 x g)



AEX or CEX column

12 x 75 mm test tube

2.0 ml collection tube
(only included in 2 pk)



Micro Bio-Spin 6 column, Tris

Fig. 2. Aurum ion exchange mini kit components

Section 5
Guidelines for Aurum Ion Exchange Columns

- All samples should be clarified before application to Aurum ion exchange columns. This can be accomplished using a 0.45 µm syringe filter or by centrifuging the sample at 10,000 x g for 5 min.
- For effective use of the Aurum ion exchange columns, the samples need to be adjusted to the approximate pH and ionic strength of the respective binding buffers. This can be accomplished in several ways. Depending upon the buffer of the sample, it may be sufficient to dilute the sample in the Aurum binding buffer. A second method would be desalting and buffer exchange using desalting columns. For small sample volumes, use Micro Bio-Spin or Bio-Spin desalting columns, and for larger volumes use Econo-Pac cartridges. A third alternative would be dialysis against the corresponding binding buffer.
- Sample loading will depend on the protein concentration of the sample and the protein capacity of the Aurum AEX or CEX column.
- If necessary, dilute samples can be concentrated using a SpeedVac concentrator or a lyophilizer.
- Knowledge of the pI of a protein of interest allows selection of resin type and buffer pH for either selective binding to or release from the resin.
- See Table 3 for buffer recommendations for the different Aurum AEX and CEX columns.
- Serum samples — Serum can be diluted 1:4 in Aurum binding buffer and then 0.4–0.6 ml applied to the ion exchange column. Using the recommended buffers, albumin will pass through the CEX column while binding to the AEX column. To selectively remove albumin and IgG from serum prior to ion exchange chromatography, use the Aurum serum protein mini kit (catalog #732-6701), which can remove >90% of these two major proteins. To remove only the albumin, use Aurum Affi-Gel® Blue columns (catalog #732-6712).
- *E. coli* lysate — For a typical *E. coli* lysate, dilute the lysate 1:4 in the appropriate binding buffer and load 0.4–0.8 ml on the ion exchange column.
- Tissue culture samples — Clarified tissue culture samples should be diluted or the buffer exchanged in binding buffer. For removal of bovine serum albumin added to culture media, use Aurum Affi-Gel Blue columns (catalog #732-6712).

- Bound proteins are eluted by increasing the salt concentration to 1 M NaCl. For some separations, varying the pH of the elution buffer in addition to its salt concentration may be advantageous.
- Aurum AEX and CEX columns show some differences in elution characteristics when eluted sequentially with different elution volumes. Table 5 shows the protein recovery in each of the three sequential elutions from both columns. Protein is more tightly bound to the CEX column, so larger elution volumes are required.

Table 5. Protein recovery for AEX and CEX elution washes.

	Elution Volume					
	200 µl		300 µl		400 µl	
Column type	AEX	CEX	AEX	CEX	AEX	CEX
% Protein Recovery — 1st Wash	84	60	82	73	86	85
% Protein Recovery — 2nd Wash	14	31	14	23	11	13
% Protein Recovery — 3rd Wash	2	9	4	4	3	2

BSA was bound to the AEX column in 20 mM Tris, pH 8.3, and eluted with 20 mM Tris, pH 8.3, containing 1 M NaCl. IgG was bound to the CEX column in 20 mM sodium acetate, pH 5.0, and eluted with 20 mM sodium acetate, pH 5.0, containing 1 M NaCl.

- The unbound fractions from the ion exchange columns can be used directly for 2-D gel electrophoretic analysis, provided the protein concentration is sufficient. For 2-D analysis, the bound fractions will require desalting. Micro Bio-Spin 6 columns (catalog #732-6221) are recommended.
- For 2-D electrophoretic analysis, see bulletin 2651, 2-D Electrophoresis for Proteomics: A Methods and Product Manual.
- Fractions can be analyzed for protein using the Bio-Rad protein assay (catalog #500-0006, Bradford method) or the *DC* protein assay (catalog #500-0116). See bulletin 1069, Colorimetric Protein Assays.
- If pI of protein is unknown — We recommend running an IEF gel (catalog #345-0072) to determine its pI. If the sample is not pure, we recommend visualizing the protein using an activity assay or identifying the protein with an immunoblotting assay. If the amino acid composition is known, the theoretical pI can be computed using databases available on the Internet, such as at www.expasy.org.

Section 6
Protocol

Please read the section “Guidelines for Aurum Ion Exchange Columns” before proceeding.

1. Place an ion exchange column in a 12 x 75 mm test tube and allow the resin to settle for at least 5 min.
2. Remove the cap and break off the tip from the bottom of the ion exchange column. Return column to test tube.
3. Start gravity flow in the column and allow residual buffer to drain from the column (approximately 2 min). If the column does not begin to flow, push the cap back on the column and then remove it again to start the flow.
4. Once the residual buffer has drained, wash the column with 2 x 1 ml of the appropriate Aurum AEX or CEX binding buffer using gravity flow. Allow each wash to pass fully through the column and drain.
5. After the last wash, place the column in an empty 2.0 ml collection tube and centrifuge for 10 sec at 1,000 x g in a microcentrifuge to dry resin bed and frit. **Do not overdry resin bed and frit.** Discard the collection tube.
6. Place the column in a clean 2.0 ml collection tube labeled **“unbound”**.
7. Load the sample in the appropriate buffer onto the column and allow the sample to gravity filter through the column, collecting in the collection tube. If the column does not begin to flow, push the cap back on the column and remove it again to start the flow.
8. Place the column in another clean 2.0 ml collection tube.
9. Wash the columns with 0.3 ml of Aurum AEX or CEX binding buffer. Unbound fractions can be combined for further analysis if desired. Otherwise, unbound fractions can be discarded.
10. Place the column in a 2.0 ml collection tube and wash with 0.6 ml of the appropriate Aurum AEX or CEX binding buffer. Centrifuge column for 20 sec at 1,000 x g in a microcentrifuge, collecting the eluate in the tube. This wash can be discarded.
11. Place the column in a new 2.0 ml collection tube labeled **“bound #1”**.
12. Add 0.3 ml of the appropriate Aurum AEX or CEX elution buffer to the top of the column and centrifuge for 10 sec at 1,000 x g.
13. Wash with an additional 0.3 ml of the Aurum AEX or CEX elution buffer into the same “bound #1” tube. Centrifuge for 10 sec at 1,000 x g.

14. Place the column in a new 2.0 ml collection tube labeled **“bound #2”**.

15. Wash with 0.3 ml of the Aurum AEX or CEX elution buffer. Centrifuge for 10 sec at 1,000 x g.

Sample Preparation for IEF Using Micro Bio-Spin 6 Chromatography

Desalting Procedure

1. Invert the column sharply several times to resuspend the settled gel and remove any bubbles. Snap off the tip and place the column in a 2.0 ml collection tube. Remove the top cap. If the column does not begin to flow, push the cap back on the column and remove it again to start the flow. Allow the excess packing buffer to drain by gravity to the top of the gel bed (about 2 min). Discard the drained buffer and place the column back into the 2.0 ml tube.

2. Centrifuge for 4 min in a microcentrifuge at 1,000 x g to remove the residual packing buffer. Discard the buffer.

3. Place the column in a clean 1.5 or 2.0 collection tube. Carefully apply the sample (20–75 µl) directly onto the center of the column. Application of more or less than the recommended sample volume may decrease column performance.

4. After loading sample, centrifuge the column for 4 min at 1,000 x g.

5. Following centrifugation, the purified sample is now in the low ionic strength binding buffer. Molecules smaller than the column’s exclusion limit will be retained by the column.

6. Dispose of the used column.

Buffer Exchange

The gel in the Micro Bio-Spin columns is suspended in Tris buffer, pH 7.4. The gel matrix is compatible with most aqueous buffers. Buffer exchange can be achieved using the following procedure.

1. Follow steps 1 and 2 in the Desalting Procedure section.

2. Apply the new buffer in 500 µl aliquots. After each application of new buffer, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard buffer from collection tube. Repeat as required. Three washes result in >99% of the buffer exchanged. Four washes result in >99.9% of buffer exchanged.

3. Sample can now be applied to the column as directed in steps 3–6 in the Desalting Procedure section.

Section 7
Troubleshooting Guide

Problem	Possible Cause	Possible Solution
Low protein concentration	Low levels of protein in sample	Increase load on column
		Concentrate sample in SpeedVac
High protein concentration	High levels of protein in sample	Decrease the load applied to column
Buffer does not flow through column	Frit is clogged with sample debris	Prefilter sample prior to loading
Protein does not bind to column	Incorrect column chosen	CEX column is for basic proteins; AEX column is for acidic proteins
	Ionic detergents can prevent sample from binding	Avoid using detergents with opposing net charges to that of ion exchange column. Detergents can be removed using Bio-Beads® SM-2 macroporous beads (catalog #152-3920)
	Ionic strength too high	Dilute sample or desalt
	pH not correct	Check pH of loading buffer
Protein does not elute from column	Salt concentration too low in elution buffer	Increase salt concentration to 2 M and/or change pH
Sample is dilute after centrifugation	Excess packing buffer was not removed	Remove excess packing buffer by centrifugation prior to loading sample

Section 8
Bibliography

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- UNOsphere cation exchange support, Bio-Rad bulletin 2669
- UNOsphere S technical data, Bio-Rad bulletin 2678
- UNOsphere Q and S ion exchange media instruction manual, Bio-Rad bulletin 4110109
- Sample preparation — A guide to methods and applications, Bio-Rad bulletin 1825
- 2-D electrophoresis for proteomics: A methods and product manual, Bio-Rad bulletin 2651
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Section 9
Ordering Information

	Kits*		Columns (Bulk)*	
	2 pk	**10 pk**	**25 pk**	**100 pk**
Aurum AEX mini	732-6710	732-6705	732-6706	732-6707
Aurum CEX mini	732-6711	732-6702	732-6703	732-6704
Aurum Affi-Gel Blue mini	732-6712	----------	732-6708	732-6709
Aurum serum protein mini	732-6713	732-6701	----------	----------
Micro Bio-Spin 6, Tris	----------	----------	732-6221	732-6222

*Kits include columns, buffers, and instructions. Columns include columns and instructions only.

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