PHOEBUS SEC COLUMN

USER MANUAL

EV Scientific

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Introduction

Phoebus is a gel filtration column designed for the separation of sample components based on differences in their size. The column is dedicated to the isolation of extracellular vesicles from plant material, bacterial culturing media, or as an additional clean-up of pre-purified samples (e.g., extracellular vesicle fractionated with density gradient ultracentrifugation). For more complex matrices like serum/plasma samples or cell culturing media, the **Artemis SEC Column** (**product ID: EVS-SEC-002; EV Scientific**) is recommended.

During the separation, vesicles poorly penetrate the pores of the column bed and are eluted before salts, small molecules, and biopolymers (proteins, pectins, etc.). This mechanism enables the purification of extracellular vesicles from other components of the analyzed samples.

Phoebus is dedicated to R&D use only. The product must not be used in medical or diagnostic applications.

Instructions for use

The samples should be devoid of any residues (e.g., cells, cellular debris, large aggregates). It is recommended to filter or centrifuge samples to pellet the particulate matter before SEC separation. Sample preconcentration using ultrafiltration or ultracentrifugation should be considered before SEC purification.

The elution solutions should be filtered with a 0.22 µm filter before use. The temperature of the elution solution and the column should be adjusted to room temperature before use.

1. Carefully remove the red cap and assemble the column in the holder in a vertical position.
2. Remove the bottom cap and discard the storage solution. The storage solution might be removed using a pipette, or the column can be left until the whole storage solution flushes through.
3. Fill the upper reservoir of the column with 3 mL of elution buffer to rinse the packed bed. Wait until the whole elution buffer flows through the bed. Repeat twice to accomplish the conditioning process.

It is recommended to use PBS or solutions featuring similar pH and ionic strength as eluents. However, any other buffer in the pH range from 4 to 12 can be used.

4. Load the sample. The sample volume should not exceed 0.5 mL (greater volumes might affect the purity of fractionated vesicles).
5. Let the sample enter the column, put 1 mL of elution buffer on the upper frit, and wait until it completely flows through the column.
6. The eluates obtained in steps 4 and 5 might be discarded (void volume).
7. Put 1.5 mL of elution buffer in the upper reservoir of the column and collect the fractions. These fractions are rich in extracellular vesicles.

It is recommended to load 0.5 mL of sample/elution buffer on the upper frit and wait until the solution enters the column. Only then should another 0.5 mL of elution buffer be loaded. These steps should be repeated until all desired fractions are collected.

8. Before the next use, rinse the column with 3 mL of 0.5 M NaOH solution followed by 9 mL of elution buffer.

9. For storage, fill the column with elution buffer supplemented with 0.05% sodium azide and close with caps. Such secured columns can be stored at room temperature.

Characteristics

Recommended sample volume	0.5 mL
Recommended fraction size	0.5 mL
EV collection (fraction size: 0.5 mL)	Fractions 4 – 6
Void volume	1.5 mL
Packed bed volume	4.7 mL
EV recovery	>90%
Storage solution	Sodium azide

Elution profile

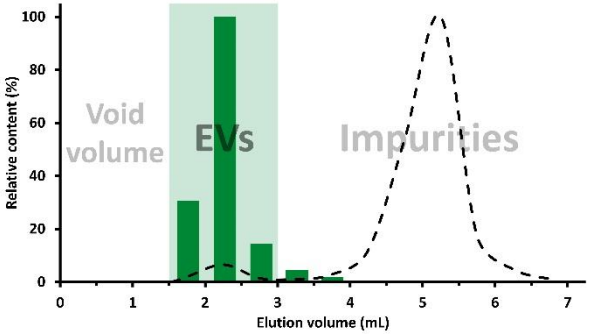

Figure 1. The elution profile obtained with a Phoebus column during the separation of plant material (*Citrus sp.* juice samples). Green bars represent the relative content of extracellular vesicles (EVs) in certain fractions. The dashed trace shows the relative protein content determined with the BCA assay.

Ordering information

Product name	Product ID
Phoebus SEC Column	EVS-SEC-001
Artemis SEC Column*	EVS-SEC-002

*Available soon.

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Ver. 1.01 | Released: 07/2025