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EVCleaner

EVCleaner: size exclusion column for the isolation of extracellular vesicles

Cat. No. **C28020001**

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Content

Introduction

Required materials not provided

Technical specifications

Disclaimer

Instructions of use

- Column equilibration
- Sample application
- Washing
- Storage
- Cleaning

Performance

Related products

Ordering information

Introduction

EVCleaner are columns working on the principle of gravity flow size exclusion chromatography to efficiently separate extracellular vesicles from unwanted components in a definite sample. This method yield highly enriched extracellular vesicles preparation without bias due to protein composition or density such as common purification techniques.

The technique allows collecting the enriched extracellular vesicles fraction from the sample within 30 minutes. This makes it a fast and easy technique unlike cumbersome ultracentrifugation-based methods. The separation on such columns is also reproducible and gentle as the elution is occurring at room temperature and under atmospheric pressure. Moreover, EVCleaner can process a wide range of sample of different nature, volume and viscosity and is well suited to process biofluids.

All these features make EVCleaner a suitable method for routine extracellular vesicles isolation.

Required materials not provided

- Laboratory stand with a grip or column holder
- 0.2 µm filtration unit
- Ultrapure water
- Dubelco's modified PBS: ThermoFisher, 14190094
- PCR-grade 1.5 ml tube
- Sodium hydroxide
- Triton® X-100
- 70% ethanol
- Micropipettes and corresponding tips: 20µl – 200µl – 1000µl
- Crushed ice
- Gloves

Technical specifications

- Bed height: 5.3 cm (mean)
- Sample volume to be loaded: 100-1000 µl
- Void volume: 3.5-4.0 ml
- Frit pore size: 20 µm
- Storage buffer: 20% ethanol
- Flow rate: 500-600 µl/min. (mean)
- Absolute pH stability: 2-14
- pH working range: 3-13
- Shelf life: 9 months if stored properly (room temperature + dark)
- Number of use: for sample with high protein content (e.g. plasma), it is recommended not to use the EVCleaner column more than 5 times. However, for sample with moderate protein content (e.g. cell culture supernatant), EVCleaner columns can be used without any problem up to 10 times if cleaned and stored following instructions described in this manual.

Disclaimer

The following technical indications regarding the elution of extracellular vesicles have been calculated for a definite model where enriched exosomes from cell culture supernatant were used.

Diagenode recommends calculating elution profile of the exosomes, at the first use of the column with the corresponding experimental model.

Sample volume and viscosity and other physicochemical parameters can significantly influence the elution of the extracellular vesicles and the following proteins.

Instructions of use

1. Column equilibration

Important consideration:

A) Use 0.2 µm filtered solutions and buffers to avoid the introduction of air bubbles in the column resin

B) Use solutions and buffers at room temperature (RT)

- Remove upper cap
- Remove bottom cap and let the storage solution penetrate into the gel
- Add sequentially with a Pasteur pipette or continuously with a peristaltic pump at least 10 ml of PBS to condition the column

Important notice: Do not let the gel run dry. The top frit must remain wet all times!

2. Sample application

- After conditioning the column, put the bottom cap on and discard all the remaining supernatant at the top of the column
- Load the sample (Sample volume: 100-1000 µl) on the column.

  Diagenode recommends first performing a pre-enrichment of the sample before purifying it with the EVCleaner column. It is particularly advised for cell culture supernatant-based sample which may not contain a sufficient amount of extracellular vesicles to further process in downstream analyses.

  For pre-enrichment instructions, please refer to the corresponding manuals :

  - CaptEV pre-enrichment reagent for cell culture supernatant (Cat. No. C28030001)
  - CaptEV pre-enrichment reagent for serum and plasma (Cat. No. C28030002)
- Remove the bottom cap and while the sample penetrates the column, immediately start collecting 0.5ml fractions

  The void volume of the column is 3.5 ml (fractions 1-7) and it is not supposed to contain any extracellular vesicles. You may collect those for further confirmation analysis or discard if sure that fractions 1-7 do not contain extracellular vesicles.
- Continue adding PBS on top of the column when your EVs containing sample has penetrated the column, in order to maintain a continuous flow of mobile phase running through the gel

- Collect carefully the 1.0 ml following the void volume (fractions: 8-9) as it contains the majority of the extracellular vesicles. Keep fractions 8-9 for further analysis.

  This eluate contains the great majority of vesicles. However, some may elute a bit later, following 0.5ml (fraction 10), but the purity of those cannot be supported as proteins begin to elute at the same time.

If the determination of an elution profile of the column is of interest, keep collecting 0.5 ml fractions until 11 ml (until fraction 22) has gone through the column. You may keep these fractions for further analysis if needed.

3. Washing

- After the collection of the fractions of interest, wash the column with at least 10 ml of water
- If the sample loaded on the column was very unpure or if the column has already been used several times, it is highly recommended to clean it before storing it
- After this step, the column should be stored in 20% ethanol at RT preferentially in a dark place (no direct sunlight) until further use

4. Storage

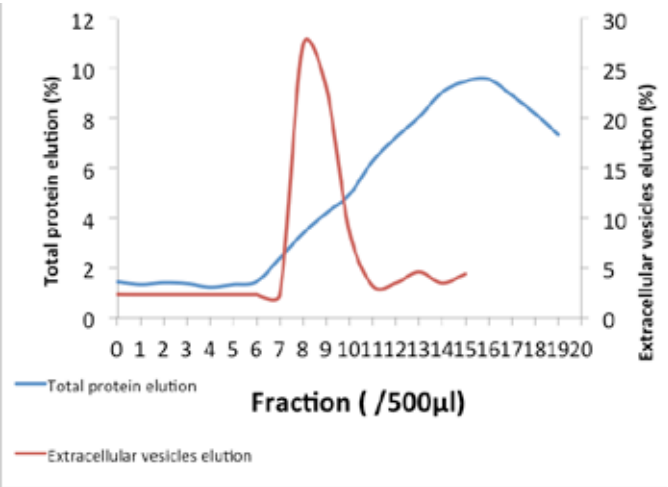
- Column can easily be stored at RT (15-25°C) in 20% ethanol and preferentially in a dark place (no direct sunlight)

5. Cleaning

- It is recommended after several uses of the column to remove precipitated or adsorbed biological material with 10 ml of 0.5 M NaOH and then another 10 ml of 0.1% TX-100
- After the cleaning, perform the wash step with 25 ml of water. The pH can also be checked prior storage with an indicator stick in order to make sure that the neutrality has been reached again. The column should be stored as described in point 4 (Storage)

Performance

The EVCleaner size exclusion column has been especially designed to enrich samples in extracellular vesicles. Thanks to a careful optimization, over 95% of soluble proteins in the sample can be discarded through the elution while a gentle recovery of all the exosomes is obtained.



This graph represents our definite model. Change in elution may occur for different experimental models.

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