AGAROSE BEAD TECHNOLOGIES

EXOSOME PURIFICATION

PROCEDURE FOR USE EXOROSE™ BEADS

DESCRIPTION

Exorose™ Beads are used for isolation of extracellular vesicles (EVs), including exosomes. The isolation is based on Size Exclusion Chromatography (SEC), separating particles based on their size as they pass through the column. Once the sample is going through the pores, the small molecules such as proteins and other contaminants become trapped in the pores and elute later than the EVs. This allows for the collection of the EVs in a pure and simple way.

Exorose™ Beads have proved their ability to separate EVs from different cell cultures and body fluids like serum, plasma, urine and saliva.

The Size Exclusion technique allow the purification of intact exosomes in a 20 minutes protocol. The resin meets the characteristics required to purify EVs while maintaining their integrity thanks to this non-invasive and inert method. Vesicles will maintain their structure and function after the purification, becoming the ideal technique for functional assays.

INSTRUCTIONS

The following summarized procedure is adapted for the purification of EVs from different biological fluids and cell cultures in gravity flow columns. The steps included in the protocol are a guideline of which the technical details may vary depending on the initial sample used.

1. Column preparation

To isolate the EVs by gravity flow, we recommend packing the Exorose™ Beads in ABT empty columns (Plastic Small Columns[CAT. No. CS-20], Plastic Columns[CAT. No. C-50], Plastic XL Columns[CAT. No. CXL-50] or Plastic XXL Columns[CAT. No. CXXL-50]). Depending on initial sample volume, there is an available range of columns from 1 ml to 60 ml total capacity. It is recommended to work with loading sample volumes around 1/20 of the total resin volume.

COLUMN	CAT. NO.	TOTAL CAPACITY (ml)
Plastic Small Columns	CS	1
Plastic Columns	C	12
Plastic XL Columns	CXL	35
Plastic XXL Columns	CXXL	60

2. Sample preparation

1. Centrifuge samples prior to loading onto the columns. It is also recommended to filter or centrifuge the biological sample to remove large particulate matter.

2. Supernatant can be concentrated before application with Amicon® filters (Merck Millipore) following the manufacturer recommendations.

3. Elimination of the preservative

1. Gently shake the bottle of Exorose™ Beads to achieve a homogeneous suspension. Immediately pipette sufficient suspension to an appropriate empty column and slowly run it down the walls of the column.

Note: It is advisable to make the addition slowly to avoid formation of bubbles. The product may be degassed before it is added to the column.

Note: Use filtered PBS to hydrate first the frits of the column if necessary.

2. Let the resin pack and repeat the previous step until the desired column height is obtained without removing the lower cap of the column.

3. The column and the sample buffer should be within operational temperature range of 18-24 ºC.

4. Size Exclusion Chromatography

1. Remove the bottom cap and allow the PBS to run through the column. Equilibrate the column with 2 – 5 volumes of filtered PBS. Add the PBS in the upper part of the column and make sure no air has been trapped.

2. Load the prepared centrifuged sample volume.

3. Allow the sample to run into the column and start collecting fractions.

Note: The elution of vesicles typically peaks at 1 ml + 0.5 ml after the void volume, for a 0.5 ml sample volume and collecting 0.5 ml volumes.

Note: The majority of EVs typically elute in 1.5 ml. There are two options depending on the customer goal, maximizing recovery collecting a larger volume or maximizing purity collecting a lesser volume. If higher purity is desired, collect only the first 1 ml.

4. After all the desired fractions has been collected, wash the column with at least 1.5 column volumes of PBS before loading another sample.

5. If storing the column for future use, flush with buffer containing a bacteriostatic agent (20% ethanol).

6. Store at 4º C.

5. Column cleaning

The sanitization and column cleaning steps may be useful when purifying a sample material different from the initial one. This switch requires removal of non-specifically bound proteins, lipoproteins and precipitated proteins contained in the column.

1. Wash with 1 column volume of 0.5 M NaOH.

EXOSOME PURIFICATION

PROCEDURE FOR USE EXOROSE™ BEADS

2. Apply several column volumes of washing buffer PBS until the pH of the eluted buffer is the same as the starting PBS pH.

ABT-EXOB-Rev.2020/A