ExoPura® Basic Exosome Purification Column Kit

Product Instruction Manual (Version 1.6)

Product Name: ExoPura® Basic Exosome Purification Column Kit

Item No. Specification: UR52180(1 T), UR52182 (5 T)

Transportation and Storage: Room temperature transportation and storage, valid for 1 year.

Product Description:

ExoPura® Basic is a highly efficient exosome purification kit developed by Shanghai Umibio Science and Technology Group for separating and purifying exosome vesicles from relatively simple biological samples with high clarification (e.g., cell supernatant, milk clarification solution, etc.). ExoPura® Basic column utilizes size exclusion chromatography to separate particles based on their size as they pass through the column containing the porous polysaccharide resin. As the sample flows through the ExoPura® Basic column under gravity, the larger particles exit first, while the smaller particles enter the resin pores, which delay their elution. The ExoPura® Basic column volume consists of solid resin (stationary phase) and liquid buffer solution (mobile phase). The particles do not chemically bind to the resin, ensuring separation stability and reproducibility. The kit offers high-speed separation, superior purification efficiency, and excellent exosome purity, making it suitable for various research applications, including: electron microscopy analysis, Nanoparticle Tracking Analysis（NTA）, nucleic acid analysis, protein analysis, cytology experiments and animal experiments.

Special note: This kit is designed for exosome purification only, not for exosome concentration. Users need to buy Umibio Exosome Extraction Kit or use other ways to concentrate the sample prior to purification.

Self-provided Material:

Sterile PBS solution (0.22 μm filter membrane filtration, room temperature); packing preservation solution: 20% ethanol (ultrasonic condition degassing for 30 min or configured and left overnight, room temperature); deionized water (0.22 μm filter membrane filtration, room temperature); iron stand; 1.5 mL EP tube; 15 mL centrifugal tube; 20 mL syringe.

Product Composition:

Component Name	UR52180	UR52182
ExoPura® Basic Column	1 T	5 T
Adapter	1 T	5 T
Regeneration Solution	60 mL	300 mL
Exosome Purification Filter	5 T	25T

Operation Procedure:

I. Sample Preparation

➢**Single sample loading volume:** 0.5 mL, equilibrate to room temperature before load the sample.

➢ **Sample Concentration:** It is recommended to use Umibio Exosome Extraction and Purification Kit (*cell supernatant*) [Item No. UR52121], Umibio Exosome Extraction Kit (*milk*) [Item No. UR52146] to concentrate the sample by more than 100-fold, or concentrate the sample by more than 50-fold using ultra-isolation, tangential flow, 100 kD ultrafiltration, etc.

Note: Use ultra-isolation with caution, it may lead to protein clustering affecting resolution.

➢**Recommended BCA concentration:** Cell supernatant with 10% exosome-free serum> 3 μg/μL, serum-free cell supernatant > 1 μg/μL, milk clarification solution > 3 μg/μL.

Note: The ExoPura® Basic column primarily removes small particles like proteins and free nucleic acids, and since the purification process dilutes the sample, ensure sufficient vesicle quantity; for electron microscopy, recommended original sample particle number concentration should exceed 8.0E+10 particles/mL.

II. Sample Pretreatment

1. Concentrate the sample using the recommended method.
2. Filtration: Use a 0.22 μm filter membrane to remove large particles from the crude exosomes.

Note: Use Umibio Exosome Extraction and Purification Kit (*cell supernatant*) [Item UR52121] and Umibio Exosome Extraction Kit (*milk*) [Item UR52146] for extraction, and the sample purified by EPF column does not require 0.22 μm filter membrane filtration.

3. Prepare PBS: Use freshly filtered PBS (0.22 μm) to avoid contamination and introduction of large particles, and make sure
the temperature of PBS matches the temperature of the column (18~25˚C).

Note: Preferably use freshly dispensed PBS filtered through a 0.22 μm filter membrane on the same day, or purchase pre-filtered PBS to avoid microbial and particulate contamination. If you use PBS solution stored in the refrigerator at 4°C, **it must be equilibrated to room temperature before use**, otherwise it may introduce a large number of air bubbles in the column and affect the separation effect.

III. Column Equilibration and Column Cleaning

1. Make sure the ExoPura® Basic column is within the operating temperature range of 18~25˚C before the experiment. Do not remove the column cap until the operating temperature range is reached.
2. Remove the black rubber cap from the top cap first to equalize the air pressure and then remove the top cap.
3. Mount the ExoPura® Basic column vertically on the iron stand and place the waste collection tube (either centrifugal tube or beaker) under the column for later use.
4. Discard the packing preservation solution above the sieve plate （remove the bottom cover, allowing the solution to drain naturally or remove it by pipette）.
5. After the packing preservation solution runs out, wash the upper chamber of the ExoPura® Basic column 2~3 times with PBS, and then add 20 mL of PBS to clean the packing inside the column (PBS can be added in several portions, or connected to an adapter, and the flow-through time is about 1.5~2.5 min per mL). When all PBS is in the column, the liquid will stop flowing.

Note: Adapter Usage: Connect a 20 mL syringe barrel above the green adapter.

6. Load the sample directly after the PBS has run out, or add 2 mL of PBS and cover with the bottom cap for later use.

Note: When using the adapter, once the syringe is empty and approximately 2~3 mL of buffer solution remains in the column, remove the adapter, wait for the remaining PBS to run out, and then either proceed with sample loading or add 2 mL of PBS and cover with the bottom cap for later use.

IV. Exosome Collection

1. Preparation before sample loading:

Use the recommended method to concentrate and filter the sample, prepare several labeled 1.5 mL EP tubes for use.

2. Add 0.5 mL of sample: As soon as PBS is no longer flowing, add 0.5 mL of room temperature-equilibrated sample to the ExoPura® Basic column sieve plate (if the sample is less than 0.5 mL, make up the amount with PBS).

Note: Avoid stopping the column flow for a long time during the process to ensure effective vesicle separation.

3. Flow through 2.87 mL of buffer solution* (void volume): When all samples are in the sieve plate and no liquid comes out of the bottom outlet, add **2.37 mL of PBS** and wait for the PBS to flow through until no liquid comes out. During this process, a total buffer solution volume of 2.87 mL flowed out (0.5 mL sample volume + 2.37 mL buffer solution)

*This volume of buffer solution is the discardable eluent before the solution containing a high percentage of purified exosomes flows out.

Note: To accurately determine the flow-through volume, add only a fixed volume of solution and wait for it to flow through until the flow stops.

4. Collect exosome fractions: Immediately prepare to collect the purified volume with a new EP tube, add 0.5 mL of PBS above the sieve plate in batches, collect 2~3 fractions at 0.5 mL/fraction (Purity is best for the first 2 fractions i.e., 1 mL volume, and it is recommended to combine the first 3 fractions, i.e., 1.5 mL volume), and each time wait for the volume to flow through until the flow stops.

5. Exosome filtering: Transfer the collected exosome fraction into the upper chamber of the Exosome Purification Filter (EPF column) and centrifuge at 3,000 × g (~6,200 rpm*) for 10 min at 4°C. After centrifugation, collect the liquid at the bottom of the EPF column tube, which is the filtered exosome (Note: The EPF column is not reusable).

*Converted by a small centrifuge with an effective centrifugal radius of approximately 7 cm (≤2 mL centrifugal tube).

6. Determine the particle concentration and protein concentration of the collected product. If required）, use

100 kD ultrafiltration tubes for further concentration and enrichment of the collected products (optional).

V. Column Regeneration and Storage

1. **Column regeneration:** After collecting the required volume, wash with 10 mL of regeneration solution, then wash with 20 mL of PBS, and proceed with the second sample loading.

2. **Column storage:** If you want to store it for future use, first clean it with 10 mL of regeneration solution, and then clean it with 20 mL of PBS, 20 mL of deionized water, and 20 mL of 20% ethanol in sequence. After cleaning, cover it with a bottom cap, pour in the preservation solution (20% ethanol), cover it with a top cap and seal it for storage.

Note: The regeneration solution is alkaline. Avoid adding deionized water or 20% ethanol directly after cleaning the regeneration solution to balance the purification column, to prevent salt precipitation in the resin bed and damage to the column.

Deionized water cleaning can further wash away the salt generated during the equilibration process and prevent the salt from crystallizing in direct contact with 20% ethanol. The cleaning time for each step can be estimated at 1.5~2.5 min per ml of liquid flow-through to avoid excessive drying of the packing after the liquid in the column is drained.

Precautions:

1. When the exosome fraction is used for subsequent high-throughput sequencing or other histological analysis, in order to avoid cross-contamination, it is recommended to use a new column.

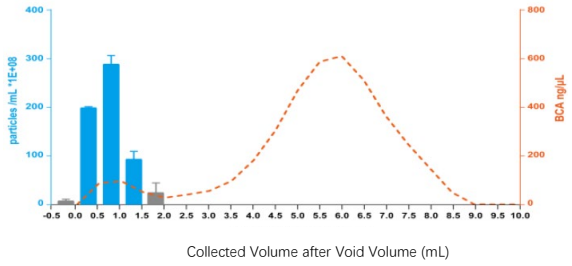
2. Salt generation during column regeneration is inevitable. Salt precipitation can significantly compromise the performance of the column packing. Therefore, it is recommended that each column be used no more than 5 times to maintain optimal separation efficiency and reproducibility.

3. Please note that the column should not be dropped from a high place or hit during the process, so as not to cause the column bed to be broken.

4. The regeneration solution is alkaline and corrosive, so please use it carefully!

5. This product is for life science research only, and medical diagnosis or other purposes are prohibited!

Result Display:

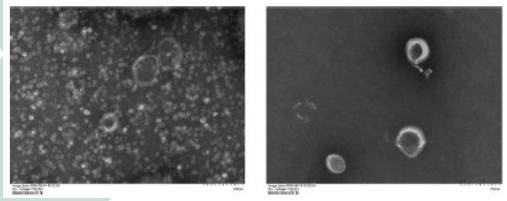


*The blue bar graph shows the recommended collection fractions for the vesicles, consisting of the three 0.5 mL fractions after the void volume.

Case: ExoPura® Basic Purification of 293T Cell Supernatant (with 10% Exosome-free Serum)

Sample Pretreatment: Concentrate 50 mL of supernatant to 0.5 mL using the Umibio Exosome Extraction and Purification Kit (*Cell Supernatant*), pass through the EPF column and prepare for sample loading.

Column Purification: Collect 1.5 mL of purified volume after collecting the void volume.



Before After