EVSecond L70 Instruction Manual

Thank you for purchasing GL Sciences' EVSecond L70.

EVSecond L70 is a size exclusion chromatography open column optimized for effective purification of exosomes.

Highly-purified exosomes can be easily collected from serum, plasma, or cell culture supernatant. To maximize the performance of EVSecond L70, read the following instructions before use.

Handling

1. When storing, do not open the aluminum bag. Store the aluminum bag in a refrigerator at 4°C. Use the product within a month to obtain maximum performance.
2. Do not drop or bump the column. Any strong impact may cause breakage of the media in the column.
3. When removing the cap from the column, make sure to gently remove the top cap in the beginning. Then, remove the plug located at the bottom of the column.
4. Before using this product, return the column to the room temperature and shake the column gently to thoroughly mix the media inside the column. Then wait until the media inside the column is COMPLETELY uniformed. It will take some time to have the media to become uniformed after shaking the column. For efficient operation, it is recommended to shake the columns in prior the operation to save time.
5. Do not remove or take the media out from column.

Typical Procedure

Please refer to the protocols shown in the enclosed paper.

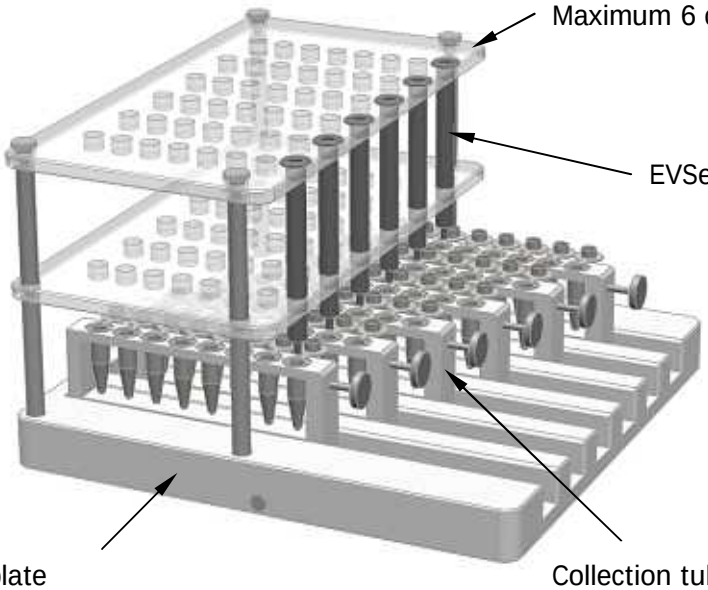
Product Information

Cat.No.	Description
5010-50450	GL-SPE EXO Fraction Rack

※ GL-SPE EXO Fraction Rack

This is a fraction rack designed for using EVSecond L70, which offers smooth column handling and fractionation.

Dimension: 300(W) x 300(D) x 189(H) mm (for EVSecond L70)



NOTE: GL-SPE EXO Fraction Rack does not include the columns and the collection tubes.

EVSecond L70 Protocol

(Extracellular Vesicle Isolation by Size Exclusion Chromatography on Drip Column)

【Sample preparation of serum sample】

1) Centrifuge the serum/plasma sample 12,000 x *g*, at 4°C for 15 minutes.
2) Do not collect any sample from the bottom precipitate and surface lipids. Only collect the sample from the middle layer/intermediate layer and collect 50 to 1500 µL.

【Preparation of EVSecond L70 column】

All procedures are required to room temperature.

1) Shake the column slowly to thoroughly mix the media inside the column. Confirm the media inside the column that is COMPLETLY uniformed. And install the column to the hanger or the stand.

※ **Important: The above procedure is to make the preservative solution to thoroughly mix with the media inside the column. If the above procedure is not operated correctly, poor fraction results will be observed.**

【Purification Procedure】

All procedures are required to be done at room temperature and gravity-flow/fall is applied to each step.

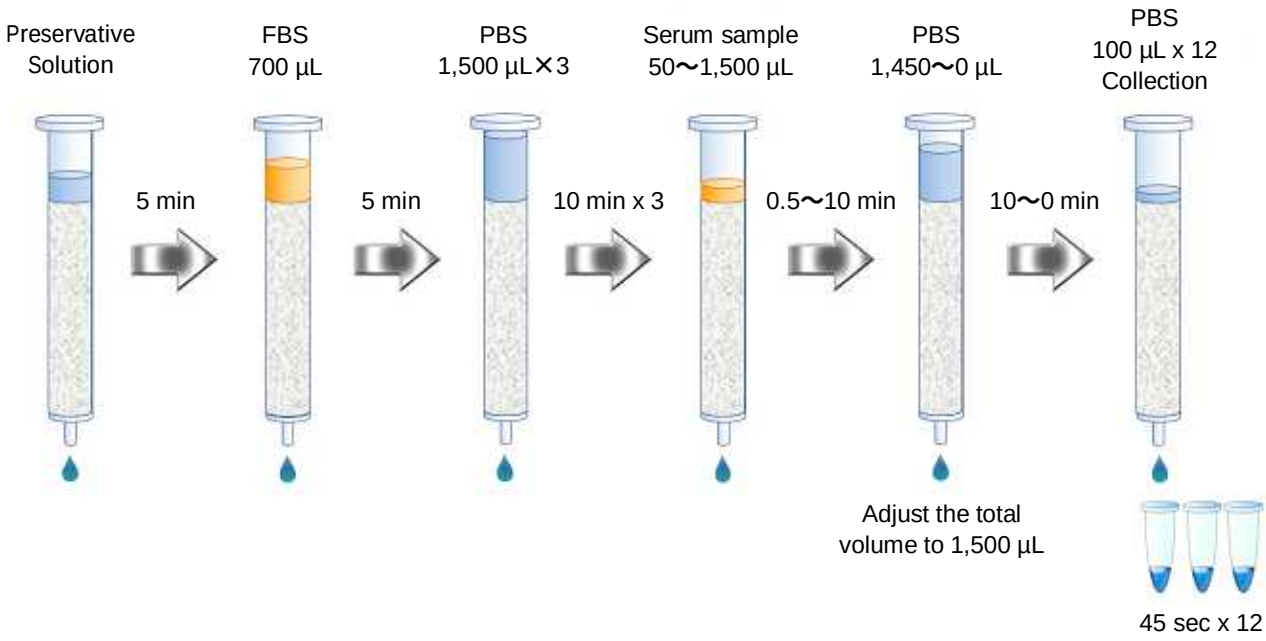
Please make sure to proceed to the next step after confirming there are no drops dropping from the column.

1) Remove the top and bottom caps from the column and discharge the preservative solution from the column. (Do not add any pressure or vibration during this step)
2) Filtrate the FBS through a 0.22 µm syringe filter. Then, load 700 µL of the filtrated FBS (or optional serum) to the column. (Blocking procedure : The blocking processing to suppress unnecessary adsorption)
3) Load 1,500 µL of PBS to the column to equilibrate.
4) Repeat the step 3) twice.
5) Gently load 50〜1,500 µL of serum sample which was prepared at the “sample preparation of serum sample” to the column and discharge the serum sample from the column.
   ※ The elution position of exosome will not change even if the loaded serum amount varies.
6) Add the PBS of from 0 to 1,450 µL slowly so that the serum sample and the total added by step 5) will be 1,500 µL.
   ※ Dispose of all solutions that were dropped from the column.
7) Finally, set the collection tube. Then, add load 100 µL of PBS, 12 times to the column and collect the appropriate fractions including exosomes.

【Confirmation Procedure】

1) Use Bradford method to confirm the elution position of serum protein.
2) Use Western blotting, ELISA method to confirm the elution position of exosome.
   ※ As a reference, please refer to the following.

【Operational flow】 (The following time is reference)

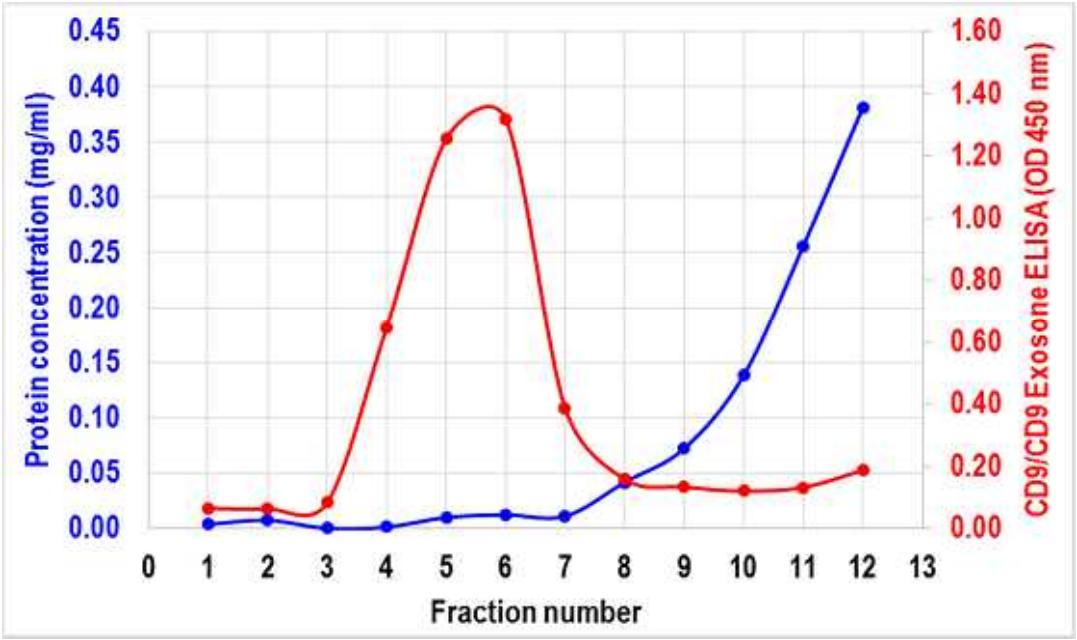


【Example Experiment】

Red：Elution position of exosome via CD9-CD9 sandwich ELISA

Blue：Elution position of serum protein via Bradford method

Fractionation/purification of exosome from 200 µL of serum sample



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