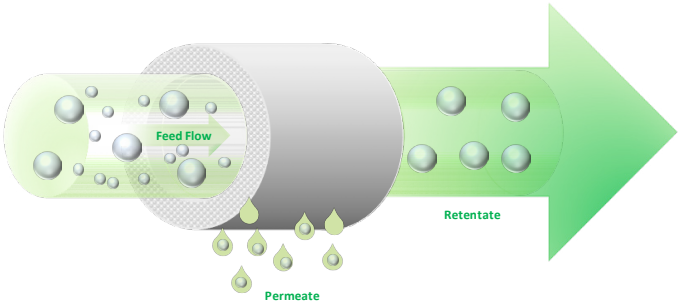
TFF-MV: Tangential flow filter for separation of medium/large EVs (>150 nm)

About TFF-MV.

TFF-MV is a filter cartridge containing polysulfone hollow fibers (200nm pores), which allows the separation of medium/large EVs from small EVs and other solutes in different fluids (cell conditioned media, urine, plasma, serum etc..).
Small EVs, proteins and other soluble molecules pass through the hollow fiber pores (permeate), whereas medium/large EVs are concentrated in the retentate. Large EVs can be easily recovered with a syringe from the filter cartridge.



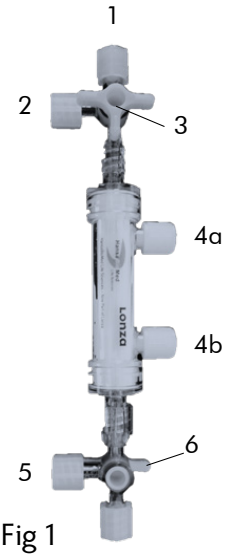


Fig 1

Specification.

1	Sample injection nozzle
2	Tangential flow filtration nozzle
3	Flow valve 1
4a	Permeate nozzles
4b	Permeate nozzles
5	Tangential flow filtration nozzle
6	Flow valve 2

Sample	Volume range	Reusable
Cell media	5 - 1000 ml	20-30 times
Urine *	5- 1000 ml	10-20 times
Plasma/serum	5-20 ml	5-10 times

Sterility: The TFF-MV is provided sterile. Once used the filter can be sterilised by Beta irradiation. Do not autoclave the TFF-MV.

Storage.

Store the device at room temperature.

EV filtration procedure.

1- Sample injection by filtration (optional).

Fig 2

- Remove the screw from the sample injection nozzle (position 1).
- Insert a syringe filter in the nozzle. The device is compatible with multiple filter types. The size of the filter to be used is user dependent.

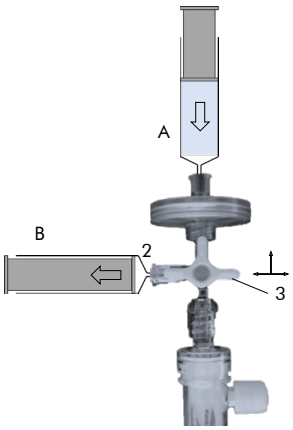
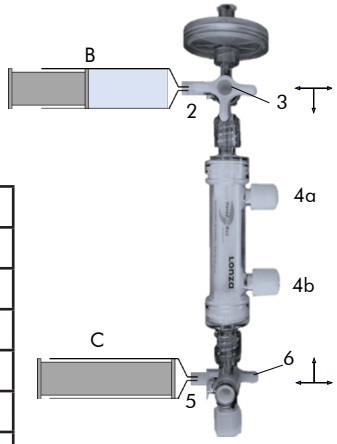


Fig 3

- Pick up the sample with syringe A and insert on the top of the filter.
- Insert a clean empty syringe in the Tangential flow filtration nozzle (position 2).
- Rotate the valve 3 to the position indicated by arrows in figure 3.
- Inject the sample into the filter by pushing the piston of syringe A. The sample passes through the filter, filling the syringe B.

2- Tangential Flow Filtration.



- Rotate the valve 3 to the position indicated in figure 4.
- Open the permeate nozzle 4b by removing the cap.
- Insert a new clean syringe (C) in position 5.
- Set a permeate collection tube under the nozzle in position 4b, as indicated in figure 4.

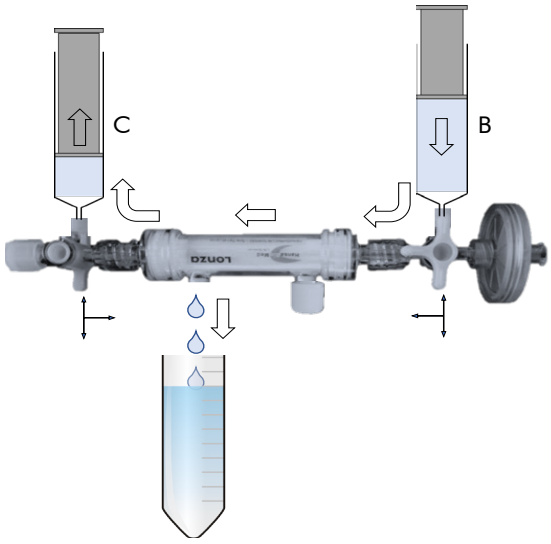


Fig 4

- Start the filtration process by pushing the syringes B and C alternatively upwards and downwards. The permeate starts to flow to the collection tube, while the medium/large EVs are getting separated in retentate.
- Continue the filtration process until syringes are completely empty and there is no more permeate running.

3- Washing residual smaller particles.

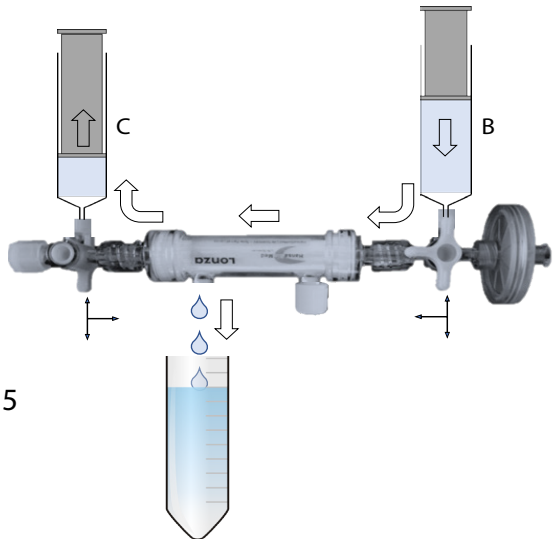


Fig 5

- While closing the flow through valve in position 3, disconnect the syringe B and fill it with PBS (~10ml).
- Reattach the syringe B, enable the flow through valve in position 3 and wash the filter with PBS until the syringes are completely empty in order to remove residual smaller particles (figure 5). Repeat the step once.

4- Recovery of medium/large EVs.

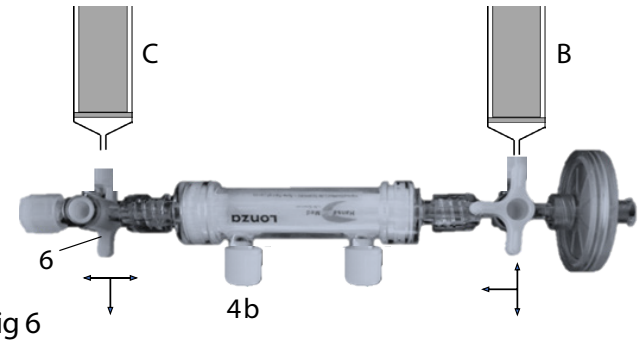


Fig 6

- In order to collect retained medium/large EVs, close the permeate nozzle in position 4b.
- Rotate the valve in position 6 as indicated in figure 6 and disconnect the syringe C.
- Load syringe C with small amount of PBS (2ml).

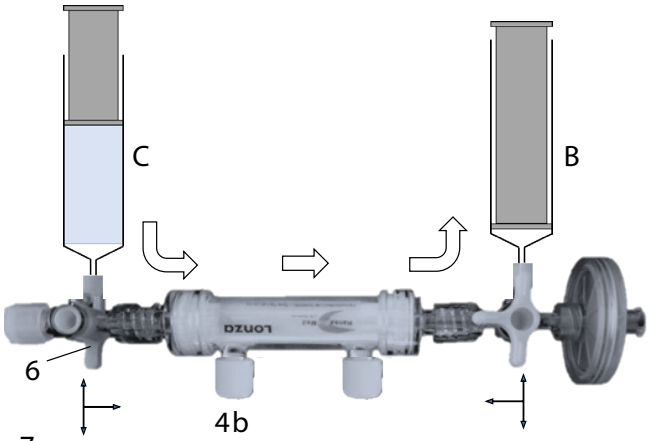
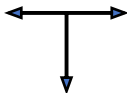


Fig7

Reattach syringe C to nozzle in position 5 and enable the flow through filter by rotating valve in position 6 as indicated in figure 7.
Inject PBS into the filter and pull up the piston of syringe B in order to collect the retentate sample.
Repeat the recovery step by injecting the air in filter, instead of PBS, in order to maximize the recovery of remaining sample.



- Rotate the valve 3 to the position indicated by arrows and disconnect the syringe B.
- Transfer the retentate sample in a clean tube.

5- Recovery of small EVs.
Collected permeate containing small EVs, soluble proteins and other small molecules can be further concentrated using HBM-LS TFF-Easy (5nm) filter.
Concentrated sample can be further purified with HBM-LS PureEV Size Exclusion Chromatography column for downstream application and analyses.

Washing procedure.

Once the filtration process is ended the filter cartridge has to be washed with abundant MilliQ water. If the cartridge is used for concentrating complex fluids it is recommended to wash as described to point 1, contamination removal.

Procedure for MilliQ water washing
Use 2 clean syringes and load one with MilliQ water (~10ml).
Connect the syringe containing water to the position 5 and the empty one to the position 2.
Close both permeate nozzles (4a and 4b) and rotate the valves 3 and 6 in order to enable the flow through the device (figure 7).
Inject the water in the device and perform washing by pushing alternatively the two syringes upwards and downwards for couple of times.
In final step, push the water in syringe B (position 2) and discard it by detaching the syringe.
Repeat the washing step at least 3 times.
Next, perform washing with MiliQ water while the permeate nozzle 4a is open.
Connect the syringe containing water (10 - 15ml) to the position 5 and the empty one to the position 2.
Flush the filter with water until syringes are completely empty.
Repeat this washing step at least 3 times.
Let the device dry at room temperature.

1- Contamination removal
A- Wash the filter with warm MilliQ water (40-50°C), carefully, applying low pressure with syringes.
B- Wash the filter with a solution of NaOH 0.5 N, then wash with at least 3 volumes of MilliQ water.

2- Optional : washing with Ethanol 96% (5 - 10 ml) can be performed for the removal of potential pyrogenic agents. Wash finally with at lest 3 volumes of MilliQ water.

HansaBioMed Life Sciences Ltd
Mäealuse 2/1
12618, Tallinn (EE)
Email: info@hansabiomed.eu
Tel: +372 6561996
www.hansabiomed.eu

Investors