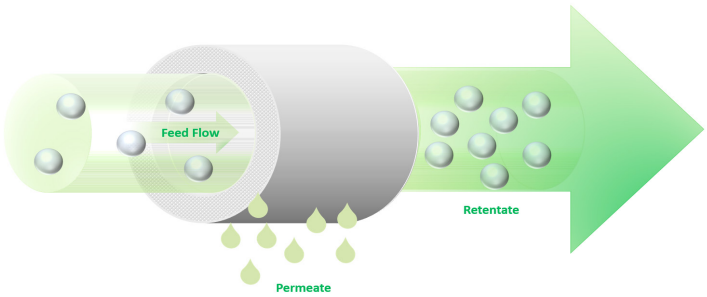
TFF-Easy: Tangential flow filtration for Extracellular Vesicle concentration

About TFF-Easy.

TFF-Easy is a filter cartridge containing polysulfone hollow fibers (5 nm pores), which allows the concentration and the removal of small proteins and molecules from diluted matrices (cell conditioned media, urine, etc..), prior to the EV purification.
Water and small molecules (< 100 kDa) pass through the hollow fiber pores, whereas EVs are concentrated in the retentate. EVs can be easily recovered with a syringe from the filter cartridge.



Specification.

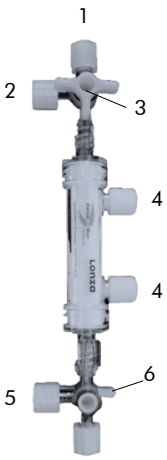


Fig 1

1	Sample injection nozzle
2	Tangential flow filtration nozzle
3	Flow valve 1
4	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

* TFF-Easy can be used for concentrating all diluted body fluids (urine, CSF, bronchoalveolar lavage etc).

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

Storage.

Store the device at room temperature.

EV concentration 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 typology. The dimension of the filter pores can be choosen by the user according to the necessity.
In order to keep all the EV subtypes it is recommended to use filter pores larger than 0.22 µm.

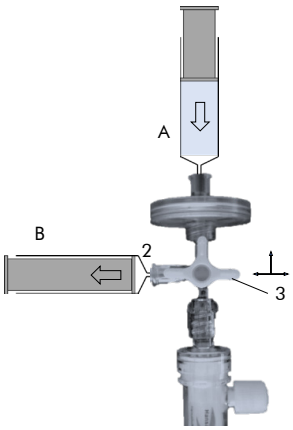
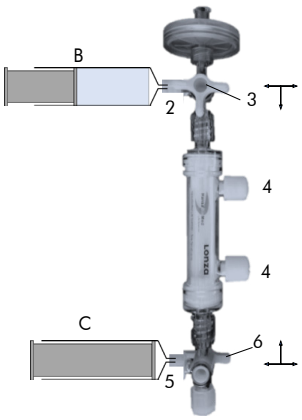


Fig 3

- 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 nozzles, removing the screws.
- Insert a new clean syringe (C) in position 5.
- Set 2 permeate collection tubes under the nozzles in position 4, as indicated in figure 4.

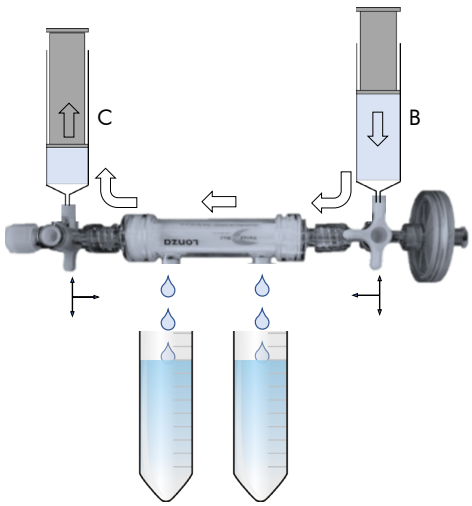


Fig 4

- Start the concentration process by pushing the syringes B and C alternatively upwards and downwards.
- Continue the concentration process until the desired volume is obtained.
- The permeate starts to flow to the collection tubes, while the retentate in the syringes contains the concentrated EVs.

3- Recovery of concentrated EVs.

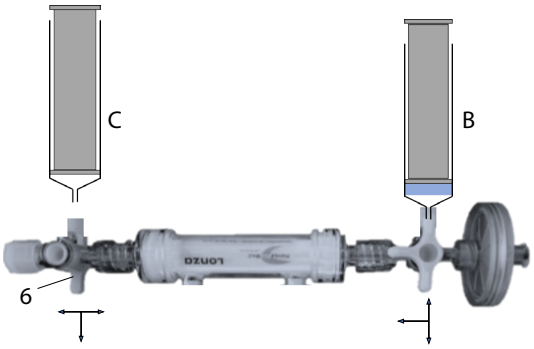


Fig 5

- In order to collect the concentrated EVs, push the concentrated products into syringe B.
- Rotate the valve 6 in the position indicate in figure 5 and disconnect the syringe C.
- Load air into syringe C.

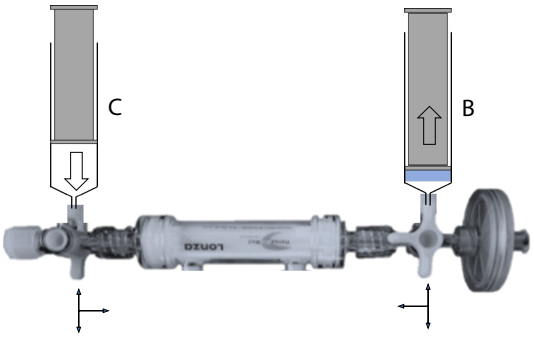
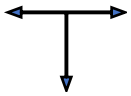


Fig 6

- Rotate the valve 6 to the position indicated in figure 6.
- Insert the syringe C and inject air into the device and pull up the piston of syringe B in order to collect all the residual volume of concentrated sample.



- Rotate the valve 3 to the position indicated by arrows and disconnect the syringe B.
- Collect the concentrated samples in a clean tube.

Fast dialysis procedure.

TFF-Easy can be used for EV dialysis and buffer exchange.

- Inject the buffer (buffer 1) containing EVs into the device as indicated in figure 4.
- Push the syringes B and C alternatively upwards and downwards until all buffer has passed to the permeate collection tubes.
- Load the syringe B with the new buffer (buffer 2) and inject into the device. Repeat the operation indicated in the point above at least 5 times.
- Concentrate the EVs in buffer 2 until the desired volume.

Example

Dialysis progress	Conductivity (µS/cm)	Particle concentration (particle number/ml)
EVs in buffer 1 (PBS 1X) 5 ml	15000	$5.8x10^{11}$
1- Removal of buffer 1 by TFF	15000	
2- Injection of buffer 2 (NaCl 100 mM) in TFF cartridge		
3- Removal of buffer 2 and buffer 1 residues	4100	
4- Injection of buffer 2		
5- Removal of buffer 2	624	
6- Injection of buffer 2		
7- Concentration of buffer 2 up to 5 ml	621	$4.9x10^{11}$

Washing procedure.

Once the concentration process is ended the filter cartridge has to be washed with aboundant MilliQ water. If the cartridge is used for concentrating complex fluids it is recommended to wash as described to point 1, Easy fouling.

Procedure for MilliQ water washing
- Use 2 clean syringes and load one with MilliQ water.
- Connect the syringe containing water to the position 2 and the empty one to the position 5
- Rotate the valve 3 and 6 in order to open the device (see fig 6)
- Inject the water in the device and continue the washing step by pushing alternatively the two syringes upwards and downwards until all the water is passed to the permeate collection tubes.
- Repeat the operation. It is recommended to wash with at least 3 volumes of water.
- Let the device dry at room temperature.

If after washing the fibers look colored or if the filter is clogged proceed as indicated:

1- Easy fouling
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- Moderate fouling
A- Prewash the filter with warm MilliQ water, as indicated above.
B- Wash the filter with a solution of NaOH 1 N, then with at least 3 volumes of MilliQ water. If the fouling persists, use a warm solution of NaOH 1N (35-40°C), then wash the filter with 3 volumes of MilliQ water.

3- Hard fouling
Prewash the filter with warm MilliQ water as indicated above.
Wash the filter with a water solution of NaClO (0.05 %), then wash with at least 5 volumes of MilliQ water.

Optional : a wash 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