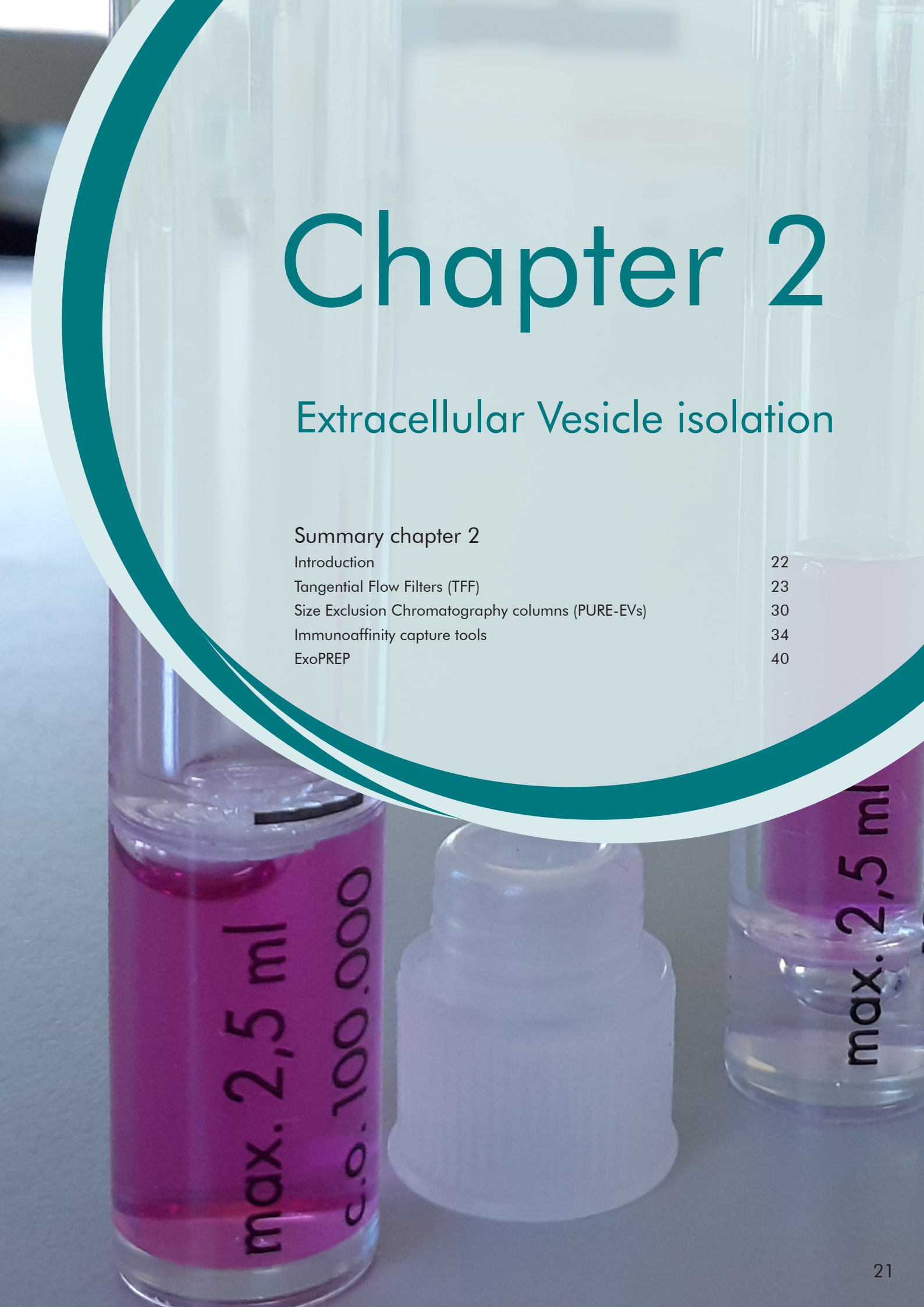
Chapter 2

Extracellular Vesicle isolation

Summary chapter 2



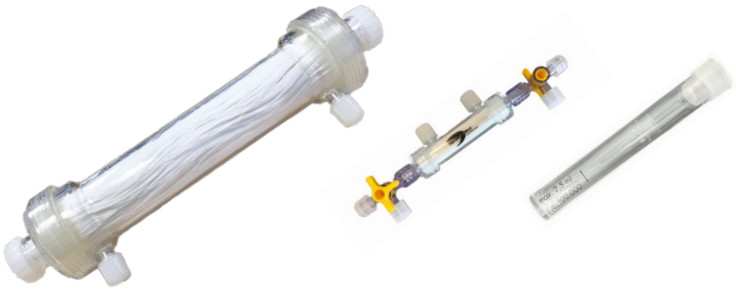
Extracellular Vesicle isolation

Introduction

Although ultracentrifugation is still widely used, it is not anymore considered the gold standard methodology for Extracellular Vesicles isolation, since it does not isolate EVs efficiently, tends to alter the vesicle shape and functionality, requires expensive equipment and is time-consuming. In more than ten years of experience, we have developed and optimized methods and tools for a fast, scalable and reproducible EV purification, for addressing the EV heterogenity and high throughput solutions for biomarker discovery.

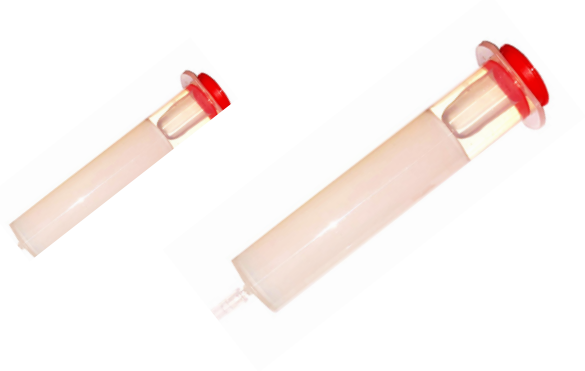
Ultrafilters

For EV size separation and medium concentration



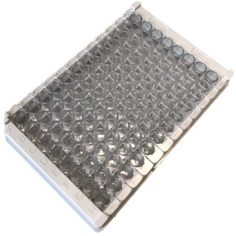
Size Exclusion Chromatography Columns

For EV purification and removal of contaminants



Immunoaffinity isolation

For biomarker screening and enrichment of EV subpopulations



Chemical isolation

For a fast and simple EV precipitation from small volume of fluids

Tangential Flow Filters for EV concentration and purification

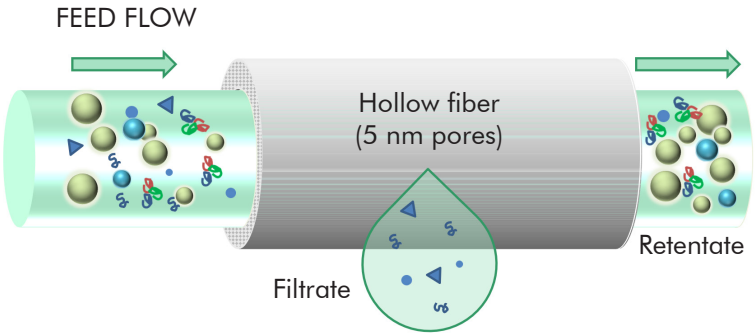
Tangential flow filtration (TFF) is a rapid and efficient method, usually used for separation and purification of biomolecules in industrial applications and can be successfully applied to isolate and separate extracellular vesicles. We provide three different typologies of filters suitable respectively for EV purification, EV concentration and Ev size-based separation.

Characteristics

- TFF-EVs, pore zise: 50 nm.
- TFF-Easy pore size: 5 nm.
- TFF-MV pore size: 200 nm.
- Suitable for manual or mechanical use.

TFF-Easy: Filter for EV concentration and dialysis

TFF-Easy is a filter cartridge in hollow fibers made of polysulfone, which allows the particle concentration and the removal of small proteins and molecules from diluted matrices (cell conditioned media, urine, etc..), prior to the EV purification.



The small dimensions of the device allow to concentrate samples from 5 ml up to bigger volumes, surmounting the limit of the TFF technique which is usable for processing big volumes of fluids.

Applications

- Concentration of diluted fluid as cell media or urine prior to EV isolation.
- Easy removal of small molecules and ions from the EV preparation.
- EV dialysis and buffer exchange.
- High efficiency of EV isolation if coupled with SEC columns.

Advantages

- Washable.
- Reusable multiple times.
- Easy to use.
- Fast concentration of EV containing matrices.

Cat. Code	Filter Volume	Quantity
TFF-Easy: EV concentration and dialysis		
HBM-TFF/1	2 ml	1 filter
HBM-TFF/5	2 ml	5 filters

References

Chetty, V. K., Ghanam, J., Anchan, S., Reinhardt, K., Brenzel, A., Gelléri, M., ... & Thakur, B. K. (2022).

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Extracellular Vesicle isolation

Applications

- Separation, concentration and recovery of large EVs (>150 nm).
- Large EV isolation from cell media, biofluids, plant extracts.
- Dialysis and desalting of large EVs.
- Suitable for large EV isolation from 5 ml of fluid.

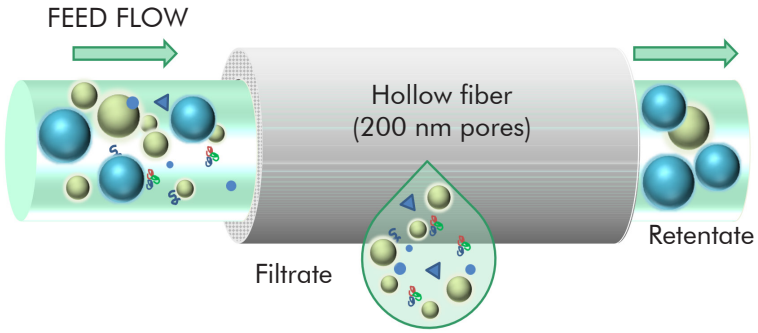
Advantages

- Washable.
- Reusable multiple times.
- Sterile
- Fast separation of EVs bigger than 150 nm.

TFF-MV: Filter for large EV separation and purification

TFF-MV is a filter able to separate large microvesicles (MVs) by size, avoiding the separation by centrifugation at 10000g, which often causes the loss of part of small EVs. TFF-MV retains vesicles larger than 150-200 nm, whereas small EVs and circulating molecules pass in the permeate. Retained MVs can be recovered with a syringe in PBS buffer, without additional purification steps.

TFF-MV is a filter cartridge made of hollow fibers with pores of 200 nm size. It can be used manually with syringes and allows the separation of large microvesicles from small EVs (< 150 nm). It works from a mimimal amount of 5 ml of fluids up to liters of fluids.

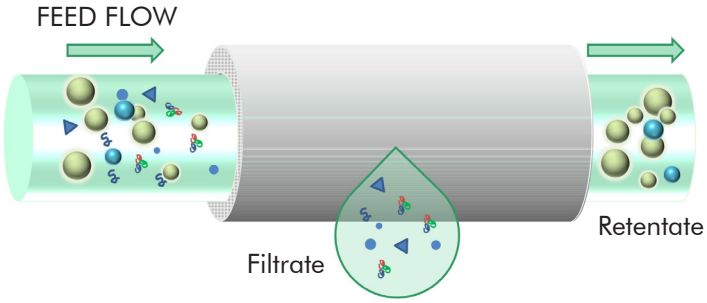


Cat. Code	Filter Volume	Quantity
TFF-MV: Separation concentration and purification of large EVs		
HBM-TFF-MV	2 ml	1 filter
HBM-TFF-MV/5	2 ml	5 filters

TFF-EVs: Fast EV purification

TFF-EVs is our next-generation filter which allows a rapid, reproducible and scalable purification of EVs, can be used on the lab bench for purifying small amount of samples (min 5 ml) or connected with a mechanical system for purifying larger volumes.

TFF-EVs is a filter cartridge made of polyethersulfonehollow fibers with pores of 50 nm size (cut off 300 kDa). The filter allows the purification of EVs and particles > 50 nm.



Avaialble in 2 sizes, the TFF-EVs Small is suitable for manual use on lab bench or under safety cabinet and allows EV purification from conditooned media or biofluids in few minutes.

The TFF-EVs Large is adapt for large scale purification and is suitable for mechanical use by peristaltic pump.

TFF-EVs Small

TFF-EVs Large

Cat. Code	Filter Volume	Quantity
TFF-EVs: Filter for EV purification		
HBM-TFF-EVs-S	2 ml	1 filter
HBM-TFF-EVs-L	30 ml	1 filter

Applications

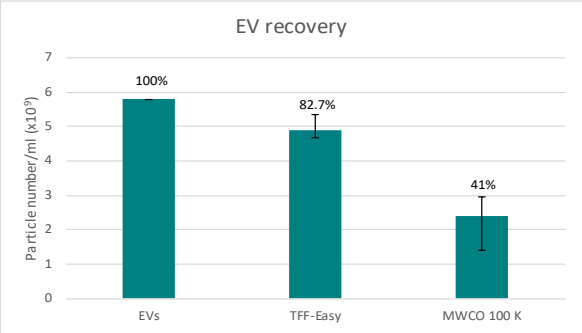
- Separation, purification and concentration of EVs from conditioned media, biofluids, plant extracts.
- Buffer exchange and removal of unbound dye.
- Depletion of FBS from bovine EVs.

Advantages

- Washable.
- Reusable multiple times.
- Sterile

Extracellular Vesicle isolation

TFF-Easy: Concentration of diluted fluids with minimal loss of EVs



1. CD81 expression in concentrated vs not concentrated CCM.

30 ug of purified EVs have been diluted in 50 ml of PBS 1x and then concentrated up to 2 ml by TFF-Easy and MWCO concentrators 100 K (Millipore). The particle concentration in the final volume has been detected by NTA (Zetaview, Particle Metrix), and compared to 30 ug of EVs diluted in 2 ml of PBS 1X. TFF-Easy allowed a recovery of approximately the 83% of the particles in solution.

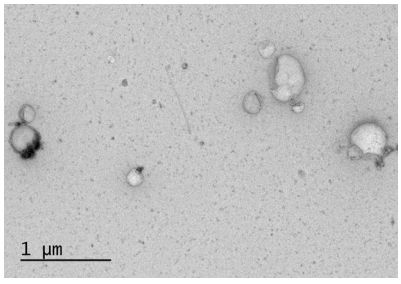
TFF-Easy allows to change the EV buffer without dialysis process

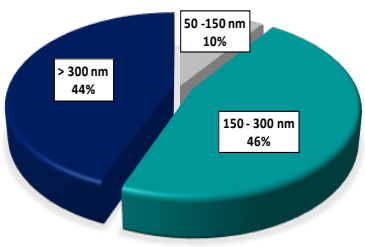
Dialysis progress	Conductivity (μS/cm)	Particle concentration (particle number/ml)
EVs in buffer 1 (PBS 1X) 5 ml	15000	$5.8x10^{11}$
1- Removal of buffer 1 by TFF	15000	
2- Injection of buffer 2 in TFF		
3- Removal of buffer 2 and buffer 1 residues	4100	
4- Injection of buffer 2		
5- Removal of buffer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

2. Process for EV dialysis with TFF-Easy.

TFF-Easy allows to dialyze EV preparation. In the process described in figure 2 we performed the EV dialysis from buffer 1 (PBS1x) to buffer 2 (NaCl 100 mM).
The TFF-Easy allows the complete removal of buffer 1, without affecting the EV concentration.

TFF-MV: Large EVs isolation, with minimal loss of small EVs

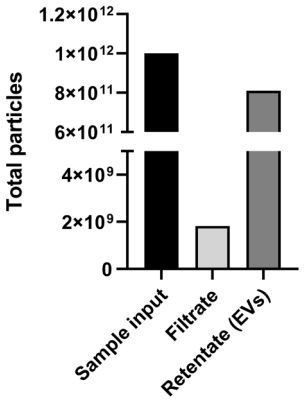




3. EM image and particle size distribution of EVs isolated with TFF-MV

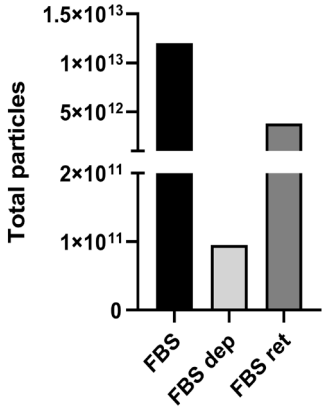
Currently, large MV are isolated or removed from small EVs by centrifugation (10000 g for 30 minutes), which also causes a massive loss of small vesicles. Moreover, different equipment (centrifuges, rotor angle, etc.) has impact on the final results. TFF-MV allows the removal of MV, provides their concentration and purification in one single step, skiping the centrifugation. The isolated MVs are pure and suitable for multiple analyses.

TFF-EVs: fast and scalable system for EV purification from CCM, biofluid, plant extracts



1x10^{12} particles of purified EVs (HBM-PEU-100) were diluted in 20 ml of PBS 1x and then injected into TFF-EVs Small. Retentate containing EVs was recovered in 5 ml of PBS 1x. The particle content of the filtrate and retentate were analyzed by NTA (Zetaview, Particle Metrix).

TFF-EVs: fast and scalable system for EV purification from CCM, biofluid, plant extracts



TFF-EVs was used to depleat the FBS from EVs of bovine origine. 50 ml of raw FBS were filtered through TFF-EVs, the filtrate contained the deplated FBS, whereas bovine EVs were recovered from the retentate in 10 ml PBS 1x buffer. All the three fractions were analyzed by NTA ((Zetaview, Particle Metrix). EV depleted FBS contains only the 1% of the total particles detected in the raw FBS.

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Extracellular Vesicle isolation

Characteristics

- Filter membrane in polyethersulfone.
- MWCO: 100 kDa.
- Concentration volume: from 2.5 ml to 0.050 ml.
- Reverse ultrafiltration (opposite direction to the centrifugal force).

Applications

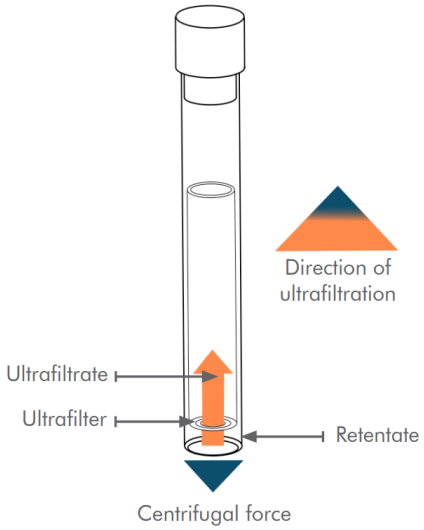
- Concentration of samples containing EVs or nanopartilces.
- Concentration of small volumes of diluted fluids.
- EV dialysis for changing buffer conditions.
- Removal of contaminants and small molecules (unbound dyes).

Advantages

- Non-stick.
- Easy recovery of concentrated material.
- Suitable for multiple washing.

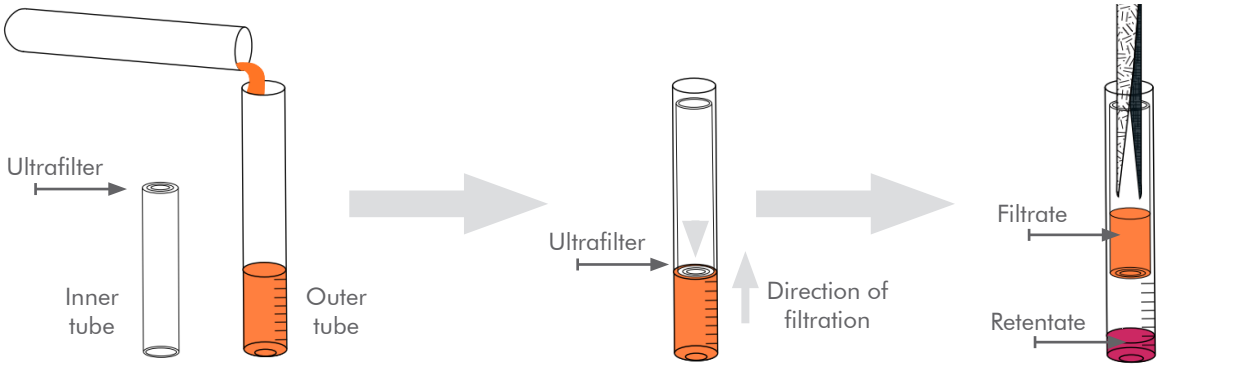
EV-Spinner: ultrafiltration concentrator

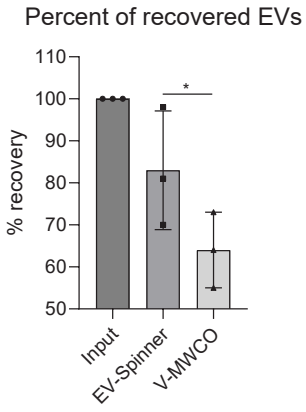
EV-Spinner is a 2.5 ml non-stick ultrafiltration (UF) concentrator to enhance the recovery of extracellular vesicle (EVs) during the concentration or buffer exchange step. The ultrafiltration works in the opposite direction to the centrifugal force, providing higher particle recovery. The low protein binding membrane (polyether-sulphone) reduces the EV loss, compared to V-shape concetrators, and the reverse design of the EV-spinner ensures that the filter does not clog.



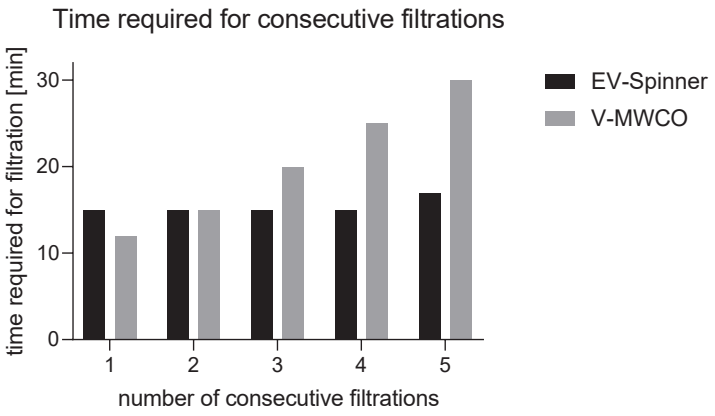
Cat. Code	Description
EV-Spinner ultrafiltration concentrator	
HBM-EVS-24	EV-Spinner 100 kDa MWCO concentrators, 24 pieces
HBM-EVS-48	EV-Spinner 100 kDa MWCO concentrators, 48 pieces

Reverse ultrafiltration for maximum recovery of extracellular vesicles and nanoparticles





Up to 98% of EV recovery was observed with 2.5 ml of EV solution concentrated up to 0.25 ml. Symbols are biological repeats, bars indicate means and error-bars are SDs.



EV-Spinner allows consecutive concentrations (from 2.5 ml up to 0.25 ml) with minimun clogging of the filter. The clogging of the filter was measured by the time necessary for concetrating a solution of EVs from 2.5 ml up to 0.25 ml.

Extracellular Vesicle isolation

Characteristics

- New gel matrix for EV purity improvement.
- Purification up to 20 ml volume of fluid.

PURE-EVs Size Exclusion Chromatography Columns

Size Exclusion Chromatography (SEC) is an efficient method for isolating and purifying Extracellular Vesicles (EVs) from different fluids, not affecting the original shape and functionality of the vesicles. We have developed a set of SEC columns which allow the EV purification from small (100 µl) and large volumes (up to 20 ml) of fluids. The EV purification process with PURE-EV columns is very fast, taking approximately 15 minutes of time.

Applications

- Extracellular vesicles isolation from cell media, biofluids and plant extracts.
- Purification of EVs from contaminants.
- Dye excess removal post EV labeling process.

Cat. Code	Volume	Columns
PURE-EV: Size Exclusion Chromatography columns		
HBM-PEV-5	500 µl - 2 ml	5 Columns
HBM-PEV-10	500 µl - 2 ml	10 Columns
miniPURE-EV: Size Exclusion Chromatography columns		
HBM-mPEV-10	100 µl - 500 µl	10 Columns
HBM-mPEV-20	100 µl - 500 µl	20 Columns
maxiPURE-EV: Size Exclusion Chromatography columns		
HBM-mxPEV-3	1 ml - 20 ml	3 Columns
HBM-mxPEV-6	1 ml - 20 ml	6 Columns

Advantages

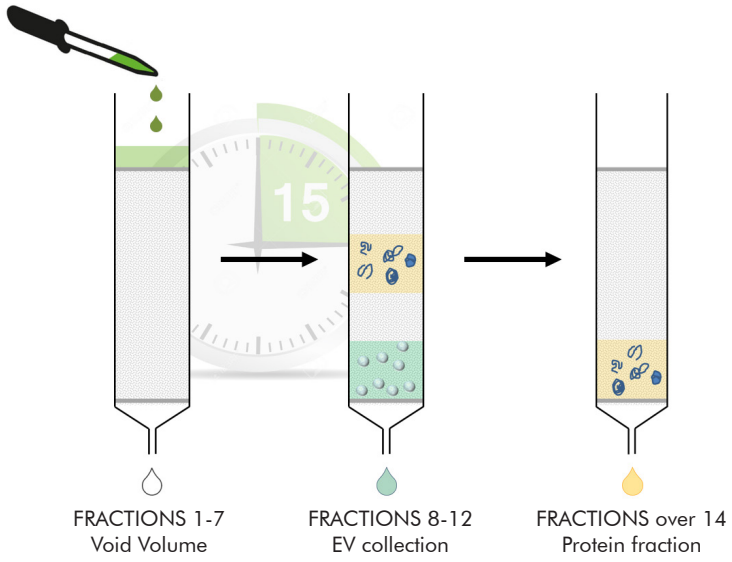
- Easy and fast protocol (turnaround time approximately 15 minutes).
- Isolate EVs from small sample volumes.
- Reusable up to 5 times.
- Long term stability at 4°C.

Maxi-PURE-EVs
Code: HBM-mxPEV
Volume: 2 - 20 ml

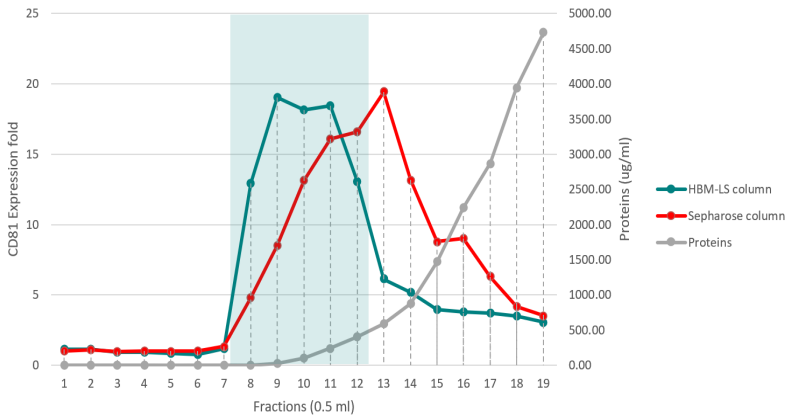
PURE-EVs
Code: HBM-PEV
Volume: 0.5 - 2 ml

Mini-PURE-EVs
Code: HBM-PEV
Volume: 0.1 - 0.5 ml

PURE-EVs: isolation of highly pure extracellular vesicles in approximately 15 minutes

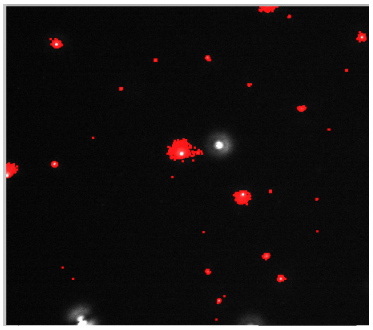


PURE-EVs column was rinsed with 1 ml of cell conditioned media from HCT116 cells, preconcentrated with TFF-Easy 10 fold. 24 fractions (500 µl each one) have been collected and analyzed by ELISA ExoTEST™ assay (see section 3, ExoTEST quantification kit) and by BCA test for determining EVs and total protein content. Results were compared with a column filled with Sepharose CL2B (GE Healthcare). EVs are eluted in fractions 8 - 12, whereas the peak corresponding to protein fraction starts from fraction 14 (Fig 4).

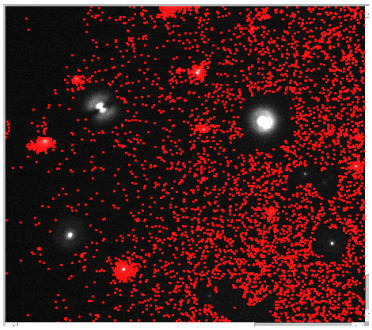


4. EV purification with PURE-EVs column (green line) vs column filled with Sepharose CL2B

mini-PURE-EVs: optimal method for removing the dye excess post EV labeling



5. Dye excess removed by mini-PURE-EVs



6. Dye excess not removed

10 µg of purified EVs from HCT116 cells were labeled by the membrane dye Cell Mask Green. The excess of the dye has been removed from the EV preparation using a mini-PURE-EVs column. The background removal has been detected by NTA with Zetaview (Particle Metrix) (Fig 5,6).

Extracellular Vesicle isolation

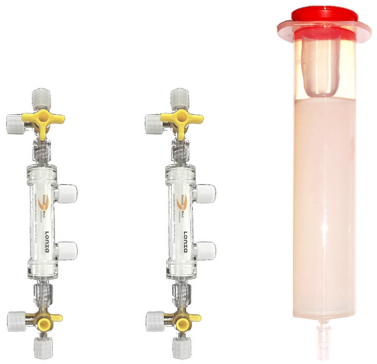
Applications

- Separation and purification of both large (> 150 nm) and small EVs.
- EV concentration and buffere exchange.
- Suitable for conditioned media and diluted biofluids (Urine).

PURE-EVs COMBO kits: EV purification from diluted fluids

PURE-EVs PLUS and PURE-EVs COMPLETE are kits which combine the ability of the TFF filters to concentrate diluted fluids to the capacity of the PURE-EVs columns to purify EVs from circulating proteins. The Combo kits are the perfect solution for people who isolate EVs from fluids as cell conditioned media or urine and want to obtain a high recovery and separation of small and large vesicles.

PURE-EVs COMPLETE: Double TFF and SEC for scalable and reproducible EV isolation and size fractionation from diluted fluids.



TFF-MV TFF-Easy Maxi-PURE-EVs

PURE-EVs PLUS: TFF-Easy and SEC combination for EV isolation and size fractionation.

TFF-Easy Maxi-PURE-EVs

Cat. Code	Volume	Columns
PURE-EV COMPLETE: Complete EV purification and fractionation		
HBM-PEV-5	500 µl - 2 ml	5 Columns
HBM-PEV-10	500 µl - 2 ml	10 Columns
PURE-EV PLUS:		
HBM-mPEV-10	100 µl - 500 µl	10 Columns
HBM-mPEV-20	100 µl - 500 µl	20 Columns

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Extracellular Vesicle isolation

Immunoaffinity isolation of Extracellular Vesicles

Addressing the EV heterogenity is becoming an important issue, in particular for the different roles that Extracellular Vesicles have in pathological processes as cancer, infection, or neurodegenerative diseases.

HBM-LS provides pre-coated ELISA Immunoplates and Latex or Magnetic Immunobeads for the capture and enrichment of total or specific EV-subpopulations.

Applications

- Multiple profiling of EV markers from a single sample or screening of a large number of samples.
- EV capture and quantification from human biofluids (plasma, serum, urine, saliva).
- Suitable for nucleic acid extraction from immunocaptured EVs.

Immunoplates for EV capture and isolation

HBM-LS Immunoplates are 96 multiwell plates covalently pre-coated with specific EV-binding antibodies allowing the capture and isolation of vesicles from different sources (cell supernatant, human plasma, serum, urine and saliva). We developed different types of plates for capturing the total or for enriching specific EV subpopulations (tumoral, neural, glial derived). Plates are blocked and stabilized for long-term storage.

Advantages

- Ready to use.
- Long term storage (up to 2 years).
- No EV pre-purification required
- Small amount of sample required (100 µl per well).
- Flexibility in designing a multiplexing assay.
- Open platform for customized coating solutions.

Immunoplates for Total EV capture and isolation

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for CD9 positive Extracellular Vesicles capture			
HBM-POS-CC/T1	Transparent	Anti Human CD9 Mouse Monoclonal	Human Plasma, Serum, Urine
HBM-POS-CC/W1	White		
ELISA Immunoplate for CD63 positive Extracellular Vesicles capture			
HBM-POC-CC/T1	Transparent	Anti Human CD63 Mouse Monoclonal	Cell conditioned media, Biofluids
HBM-POC-CC/W1	White		
Custommade Immunoplate			
Plates can be covalentely coated with EV binding antibodies, choosen from our antibody list or sent by customers. Plates are available in transparent and white format.			
Storage conditions			
Unopened: 2 years, stored at 4°C. Opened: 6 month stored at 4°C			
Material amount			
100 µl of sample per well. Whole human plasma and serum can be loaded for vesicle capture. Using urine and cell media, it is recommended to concentrate the sample 10 folds, before loading the sample on the plate.			
Packaging information			
Immunoplates are individually sealed in an opaque aluminium ziplock bag, compliant to pharmaceutical regulations. Easy to open and reseal.			

Immunoplates for enrichment of Tumor-derived EVs

Immunoplates coated with antibodies against TM9SF4 or EpCAM, two proteins widely expressed in tumor tissues and in tumor-derived EVs.

TM9SF4: TM9SF4 is a membrane protein involved in the activation of V-ATPases in conditions of elevated intracellular concentration of H+ as a consequence of elevated fermentation of sugars (Warburg effect). Ref: Lozupone F. et al. 2015

EpCAM: EpCAM is a transmembrane glycoprotein highly expressed in rapidly growing epithelial tumors. It plays an important role in localization of EVs in numerous physiological and phatological processes. Ref: Jiang L. et al. 2017; Yu L. et al. 2013

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for TM9SF4 Positive Extracellular Vesicles capture			
HBM-PTF-CC/T1	Transparent	Anti Human TM9SF4 Mouse Monoclonal	Human Plasma, Serum
HBM-PTF-CC/W1	White		
ELISA Immunoplate for EpCAM Positive Extracellular Vesicles capture			
HBM-PTE-CC/T1	Transparent	Anti Human EpCAM Mouse Monoclonal	Human Plasma, Serum
HBM-PTE-CC/W1	White		

Immunoplates for enrichment of Neural and Glial EVs

Immunoplates coated with antibodies against EV surface antigens and indicative of neurological or glial origin.

L1CAM: L1CAM is a neural cell adhesion molecule, implicated in cell migration, adhesion and neuronal differentiation. L1CAM is highly expressed in EVs from neural origin and cna be used for enrichment of neural derived EV subpopulation. Ref: Mustapic et al. 2017

PLP1: PLP1is the major myelin protein from the central nervous system and plays an importan role in the formation and the maintenance of the myelin structure. EVs derived from glial cells are characterizec by the presence of high levels of PLP1 protein.
Ref: Frühbeis et al. 2012

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for L1CAM Positive Extracellular Vesicles capture			
HBM-PNF-CC/T1	Transparent	Anti Human L1CAM-Mouse Monoclonal	Human Plasma, Serum, Cell media
HBM-PNF-CC/W1	White		
ELISA Immunoplate for PLP1 Positive Extracellular Vesicles capture			
HBM-PGF-CC/T1	Transparent	Anti Human PLP1 Mouse Monoclonal	Human Plasma, Serum, Cell media
HBM-PGF-CC/W1	White		

Applications

- Capture and enrichment of Tumor derived EVs subpopulations.
- Suitable for nucleic acid extraction from immunocaptured EVs.
- Profiling of cancer related biomarkers.

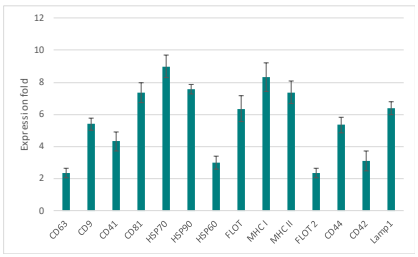
Advantages

- Ready to use.
- Long term storage (up to 2 years).
- No EV pre-purification required
- Small amount of sample required (100 µl per well).
- Open platform for customized coating solutions.

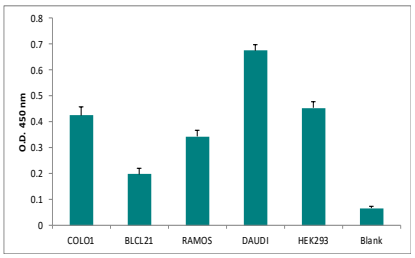
Extracellular Vesicle isolation

Immunoplates allow EV phenotyping without vesicle pre-purification steps

ELISA Immunoplates can be used for quantitative and qualitative analysis of EV-associated proteins. The plate is able to capture EVs from raw biologic material (plasma, serum, cell medium, etc.). No significant cross-reactivity is observed with soluble antigens.



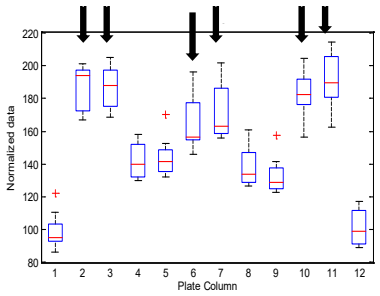
9. EV associated biomarkers analysis in a healthy donor's plasma sample.

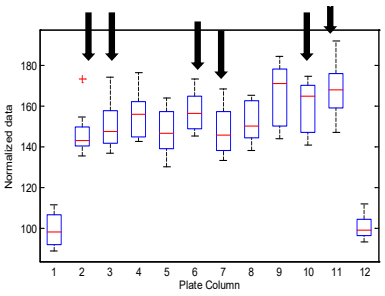


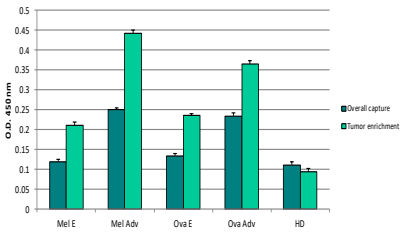
10. CD63 profiling of different cell derived EVs.

Enrichment of Tumor-derived Extracellular Vesicles

Immunoplates for tumor-derived EV enrichment (TM9SF4 coated) are able to distinguish cancer patients (black arrows) from healthy controls. The enrichment of tumor-derived EVs from cancer patient (Melanoma, Ovary) is detectable when the TM9SF4 coated plate is compared with a plate coated with CD9 (fig11).



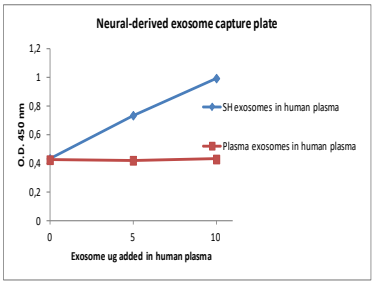




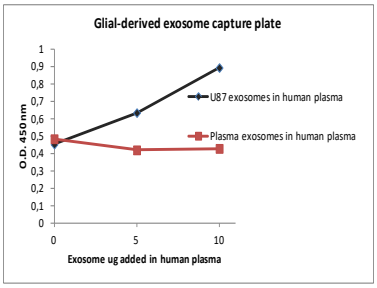
11. Enrichment in Tumor-derived EVs in early and late stage melanoma (Mel E; Mel ADV) and ovary carcinoma (Ova E, Ova ADV).

Enrichment of Neural or Glial derived Extracellular Vesicles

Immunocapture enrichment of neural- and glial-derived EVs purified from SK-N-SH and U87 cell lines and spiked in human plasma. Comparison was done with purified plasma EVs spiked in human plasma (fig 12 and fig 13).



12. Enrichemnt of SK-N-SH derived EVs spiked in human plasma from healthy donors using HBM-PNF



13. Enrichmentof U87 derived EVs spiked in human plasma from healthy donors using HBM-PGF

Immunobeads for Total EV isolation

Latex or Magnetic immunobeads are covalently coupled with antibodies against common EV surface antigens (CD9, CD63). They allow capturing EV from human biofluids (tested for plasma, serum and urine) and cell culture media without the necessity of pre-purification steps. The kit includes a Beads Elution buffer, for detaching captured EVs from antibodies and a Regeneration buffer, for regenerating the beads that can be reused ones more. Beads are sold in package of 10 reactions and are available in 2 sizes (0.4 and 1 micron diameter).

Cat. Code	Bead diameter	Antibody	Recommended for
Immunobeads for CD9 Positive Extracellular Vesicles capture			
HBM-BOLF-CC/10-04	0.4 micron	Anti Human CD9 Mouse Monoclonal	EV pheno/genotyping
HBM-BOLF-CC/10-1	1 micron		FACS analysis
Immunobeads for CD63 Positive Extracellular Vesicles capture			
HBM-BOLC-CC/10-04	0.4 micron	Anti Human CD63 Mouse Monoclonal	EV pheno/genotyping
HBM-BOLC-CC/10-1	1 micron		FACS analysis
Immunobeads for Mouse Extracellular Vesicles capture (CD9 Positive)			
HBM-BMLF-CC/10-04	0.4 micron	Anti Mouse CD9 Mouse Monoclonal	EV pheno/genotyping
HBM-BMLF-CC/10-1	1 micron		FACS analysis
Custommade Latex or Magnetic Immunobeads			
Beads can be covalentely coated with EV binding antibodies, choosen from our antibody list or sent by customers. Magnetic or Latex beads are available.			
Storage Condition			
Store the immunobeads and buffers at 4 - 8° C.			
Conditions required			
Recommended starting volume from 0.1 ml - 0.5 ml of plasma, from 0.5 ml to 1 ml of serum. Concentrated (10X) urine and cell culture medium samples are recommended prior capture according to our suggested protocol (see page 21, TFF-Easy).			

Applications

- Total EV isolation from cell culture media, human or mouse biofluids (tested for plasma, serum, urine).
- Total EV isolation from mouse biofluids (tested for plasma and serum).
- Downstream marker profiling.
- Nucleic acids extraction
- EV elution from immunobeads

Advantages

- Ready to use.
- Small sample volume of biofluid or cell culture medium.
- No ultracentrifugation or other methods for vesicle purification required.
- Supplied with buffer for EV elution from beads.
- Immunobeads can be regenerated with Beads Regeneration Buffer and reused.

2

Extracellular Vesicle isolation

Applications

- Capture and enrichment of human EV subpopulation (tumor-derived).
- Downstream EV marker profiling.
- Nucleic acids extraction
- EV elution from immunobeads

Immunobeads for Tumor-derived EV capture

Latex or Magnetic immunobeads are covalently coupled with antibodies against EV surface antigens (TM9SF4 or EpCAM) associated with pathological conditions (cancer). They allow to pull down tumor-derived EV from human biofluids, thus providing a potential new platform for the research in circulating tumor biomarker.

Cat. Code	Bead diameter	Antibody	Recommended for
Immunobeads for TM9SF4 Positive Extracellular Vesicles capture			
HBM-BTLF-CC/10-04	0.4 micron	Anti Human TM9SF4 Rabbit polyclonal	EV pheno/genotyping
HBM-BTLF-CC/10-1	1 micron		FACS analysis
Immunobeads for EpCAM Positive Extracellular Vesicles capture			
HBM-BTLE-CC/10-04	0.4 micron	Anti Human EpCAM Mouse Monoclonal	EV pheno/genotyping
HBM-BTLE-CC/10-1	1 micron		FACS analysis
Packaging information			
Immunobeads (10 reactions) are supplied with Exosome Elution Buffer, for eluting intact exosomes from beads and with Bead Regeneration Buffer, for regenerating immunobeads that can be reused twice more.			

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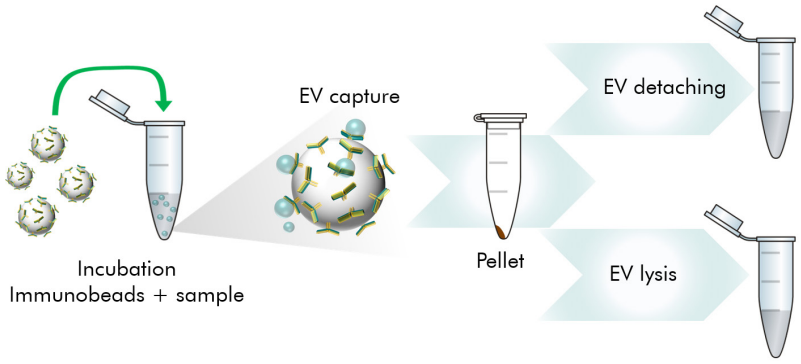
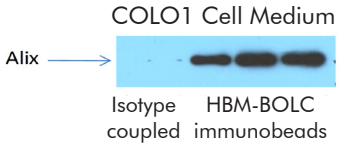
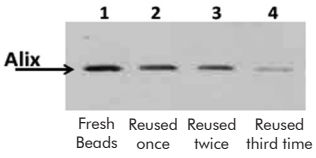
Immunobeads allow EVs capture and multiple downstraem analyses



Following incubation, beads can be recovered by centrifugation, resuspended in Laemmli buffer for SDS-PAGE and western blotting analysis (fig 14, 15) or in appropriate lysis buffer for nucleic acid analysis (fig 16). Alternatively, the vesicles can be eluted from the beads with the Elution Buffer and used for downstream applications such as ELISA or NTA. Eluted beads can be regenerated with Bead Regeneration Buffer and reused for capturing exosomes twice more (fig 15).

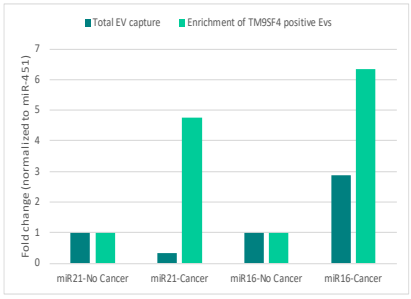


14. Alix expression by western blotting of exosomes captured on HBM-BOLC immunobeads from COLO1 cell supernatant vs isotype coupled beads.

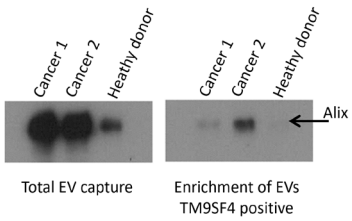


15. Western Blotting analysis of immunocaptured exosomes on beads.

TM9SF4 coated immunobeads enrich Tumor-derived EVs in cancer patient



16. Enrichment of miR21 and miR16 in cancer when TM9SF4 coated beads are used



17. Comparison of detection in WB of total and TM9SF4 positive EVs

Latex or Magnetic Immunobeads can be used for capturing EVs from raw biofluids, followed by RNA isolation. The enrichment of miRNA cancer associated (miR16 and miR21) is highly detectable when Immunobeads for capture of Tumor-derived EVs (HBM-BTF; TM9SF4 coated) are used.

Extracellular Vesicle isolation

Applications

- Single step isolation of EVs from multiple fluids
- Isolate the overall vesicles population in a sample.
- Isolated EVs are suitable for nucleic acid extraction and profiling.
- Isolated EVs are suitable for protein profiling (WB, ELISA, FACS).

Advantages

- Time and money saving.
- No ultracentrifugation required.
- Easy and fast protocol.
- Isolate EVs from small volumes of sample (as low as 100 μl of plasma).
- Easy to store and ship (4°C).

EXO-Prep: one step EV isolation reagent

EXO-Prep is a fast and convenient method of Extracellular Vesicle isolation from biofluids, cell culture supernatants, plant extracts. Isolation with EXO-Prep is based on chemical precipitation. Samples are incubated with EXO-Prep solution on ice so that EVs will precipitate following centrifugation. The obtained pellet can be resuspended in PBS 1X or deionized water. The protocol is user-friendly, time-saving (around 1 hour), and does not require capital laboratory equipment. Isolated vesicles are in particular suitable for isolation of nucleic acid associated to EVs.

Cat. Code	Volume	Reactions
EXO-Prep for Exosome Isolation from Plasma and Serum		
HBM-EXP-B5	5 ml	180 reactions Plasma, 80 reactions Serum
EXO-Prep for Exosome Isolation from Cell Media		
HBM-EXP-C25	25 ml	25 reactions
EXO-Prep for Exosome Isolation from Urine		
HBM-EXP-U25	30 ml	25 reactions

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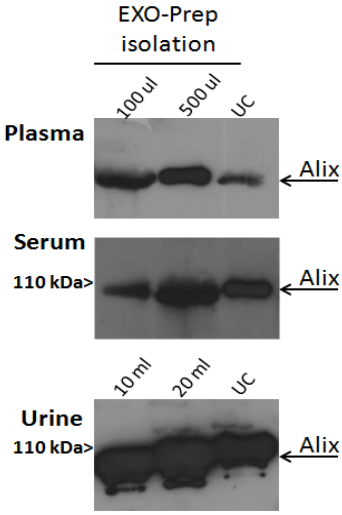
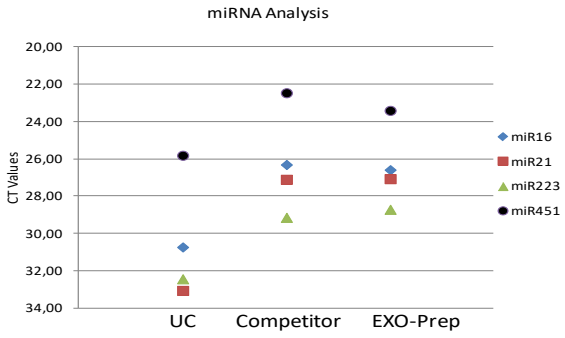
EXO-Prep isolates Extracellular Vesicles in one single step from a small volume of sample



18. WB analysis of Alix in EV lysates

EXO-Prep is able to isolate EVs form very low volume amount. EVs were isolated from 100 µl or 500 µl of human plasma, serum and 10 or 20 ml of human urine. 30 µg of protein lysates have been used for EV marker analysis (Alix) (fig 18). RNAs were extracted from EVs isolated from 500 µl of human plasma and tested for profiling of 4 different miRNA EV associated (fig 19).



19. Profiling of 4 miRNA EV associated.