2 Extracellular Vesicle Isolation Tools

Summary Section 2

HansaBioMed Life Sciences

exosomics

Extracellular Vesicle isolation tools

Ordering information

Products can be purchased directly in our on-line shop:
www.exotest.eu/online_orders/

Distributors:
www.hansabiomed.eu/index.php/distributors

Technical support

Contact us at:
info@hansabiomed.eu

Introduction

Ultracentrifugation is currently the "gold standard" methodology for Extracellular Vesicles isolation from biological fluids or cell conditioned media. However it does not isolate EVs efficiently, tends to alter the vesicle shape and functionality, requires expensive equipment and is time-consuming. To address these issues, the HBM-LS team has developed and optimized tools for isolating the total or specific EV populations in an efficient and fast way.

Tangential flow filter for EV concentration and size separation

Size Exclusion Chromatography Columns (PURE-EVs)

Extracellular Vesicle precipitation reagents

Immunoplates and Immunobeads for EV isolation

Tangential Flow Filters for EV concentration and purification

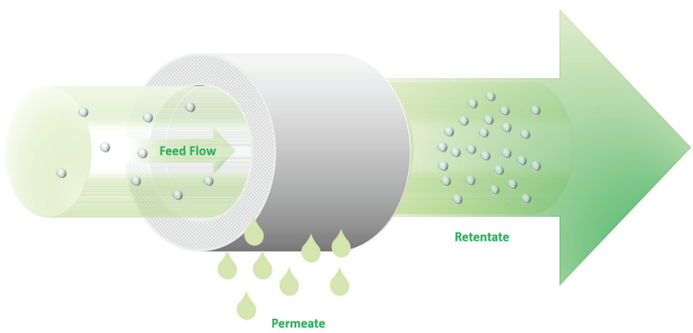
Tangential flow filtration (TFF) is a rapid and efficient method, usually used for separation and purification of biomolecules. TFF can be also successfully applied to the EV field, used for concentrating diluted fluids prior EV isolation, for EV dialysis, and for separating different EVs by size.
In this section we present 2 TFF-Filter typology.

Characteristics

- Hollow fiber filters made of polysulfone.
- 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

Our TFF-Easy is a filter cartridge in hollow fibers made of polysulfone, 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.



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.

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

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 for changing buffer conditions.
- High efficiency of EV isolation if coupled with SEC columns.

Advantages

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

Extracellular Vesicle isolation tools

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.

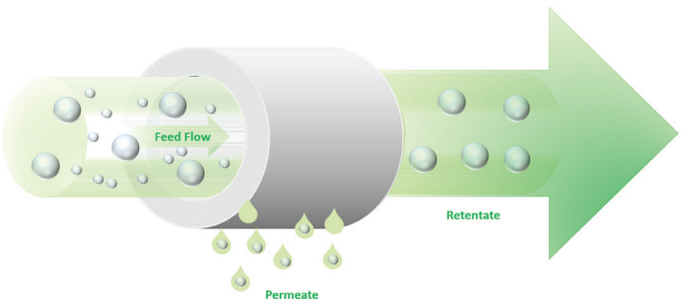
Advantages

- Washable.
- Reusable multiple times.
- Easy to use.
- Fast separation of EVs bigger than 150 nm.

TFF-MV: Filter for large EV separation and purification

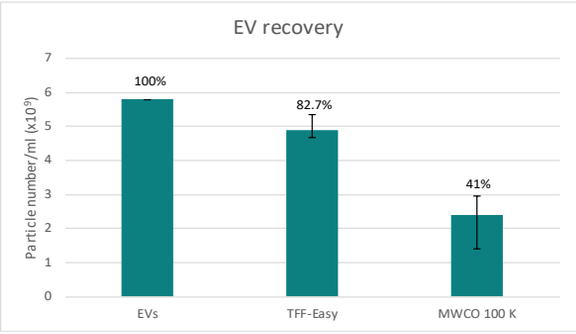
TFF-MV is a filter able to separate large microvesicles (MVs) by size, avoiding the separation by centrifugation at 10000g, which often causes the loss of part of small EVs. TFF-MV retains vesicles larger than 150-200 nm, whereas small EVs and circulating molecules pass in the permeate. Retained MVs 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

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



1. Recovery of EV post TFF-Easy or MWCO spin concentrators

30 μg 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 μg of EVs diluted in 2 ml of PBS 1X. TFF-Easy allowed a recovery of approximately the 83% of the particles in solution.

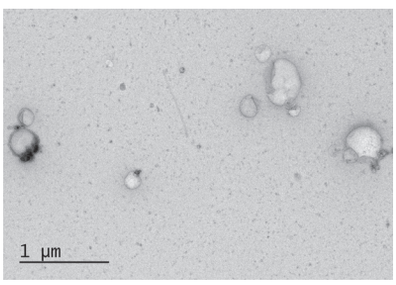
TFF-Easy allows to change the EV buffer without dialysis process

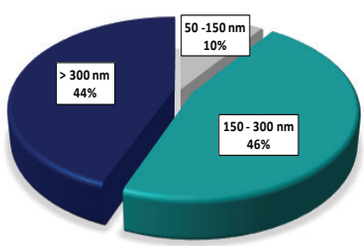
Dialysis progress	Conductivity (μS/cm)	Particle concentration (particle number/ml)
EVs in buffer 1 (PBS 1X) 5 ml	15000	$5.8x10^{11}$
1- Removal of buffer 1 by TFF	15000	
2- Injection of buffer 2 in TFF		
3- Removal of buffer 2 and buffer 1 residues	4100	
4- Injection of buffer 2		
5- Removal of buffer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

2. Process 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 EV isolation, with minimal loss of small EVs





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

Currenlty, large MVs are isolated or removed from small EVs by centrifugation (10 000 g for 30 minutes), which also causes a massive loss of small vesicles. Moreover, different equipment (centrifuges, rotor angle, etc.) impacts on the final results. TFF-MV allows the removal of MVs, their concentration and purification in one single step, skipping the centrifugation passage. The isolated MVs result is clean and suitable for multiple analyses.

Extracellular Vesicle isolation tools

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) up to larger volumes (20 ml) of fluids. The EV purification process with PURE-EVs columns is very fast, taking approximately 15 minutes of time.

Applications

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

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

Advantages

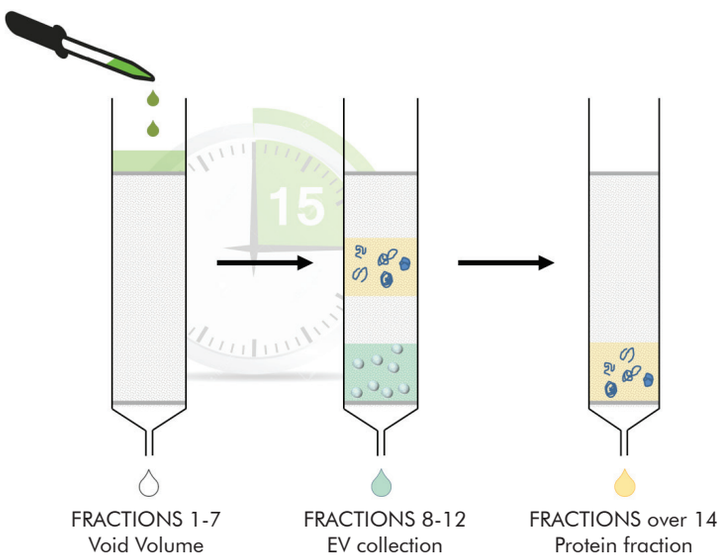
- Easy and fast protocol (turnaround time approximately 15 minutes).
- Isolate EVs from small sample volumes.
- Reusable up to 5 times.
- Long term stability at 4°C.

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

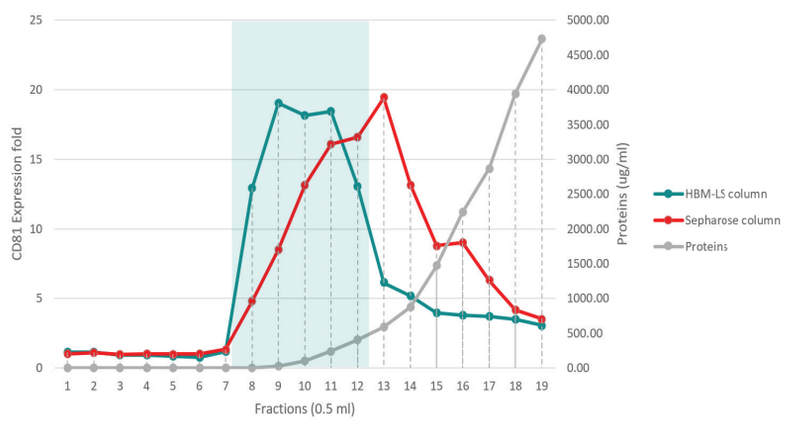
PURE-EVs
Code: HBM-PEV
Volume: 0.5 - 2 ml

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

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

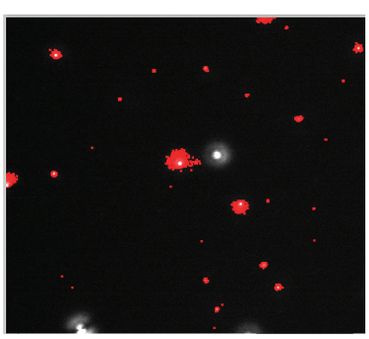


PURE-EVs column was rinsed with 1 ml of cell conditioned media from HCT116 cells, preconcentrated with TFF-Easy 10 fold. 24 fractions (500 µl each one) have been collected and analyzed by ELISA ExoTEST™ assay (see section 3, ExoTEST quantification kit) and by BCA test for determining EVs and total protein content. Results were compared with a column filled with Sepharose CL-2B (GE Healthcare). EVs are eluted in fractions 8 - 12, whereas the peak corresponding to protein fraction starts from fraction 14 (Fig 4).

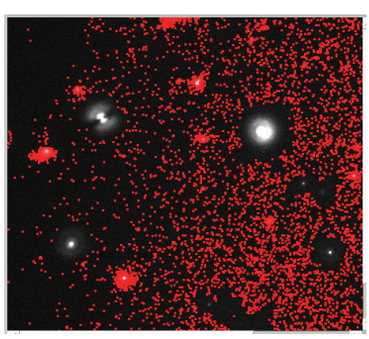


4. EV purification with PURE-EVs column (green line) vs column filled with Sepharose CL2B

mini-PURE-EVs: the optimal method for rmoving 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 tools

Applications

- Purification and separation of EVs by size from diluted fluids.
- Suitable for cell conditione media, plant extracts, diluted biofluids (urine, CSF, bronchoalveolar lavage, etc.).

Advantages

- No centrifugation steps.
- Fast turnaround time.
- Reproducible results.
- Reusable multiple times.

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.

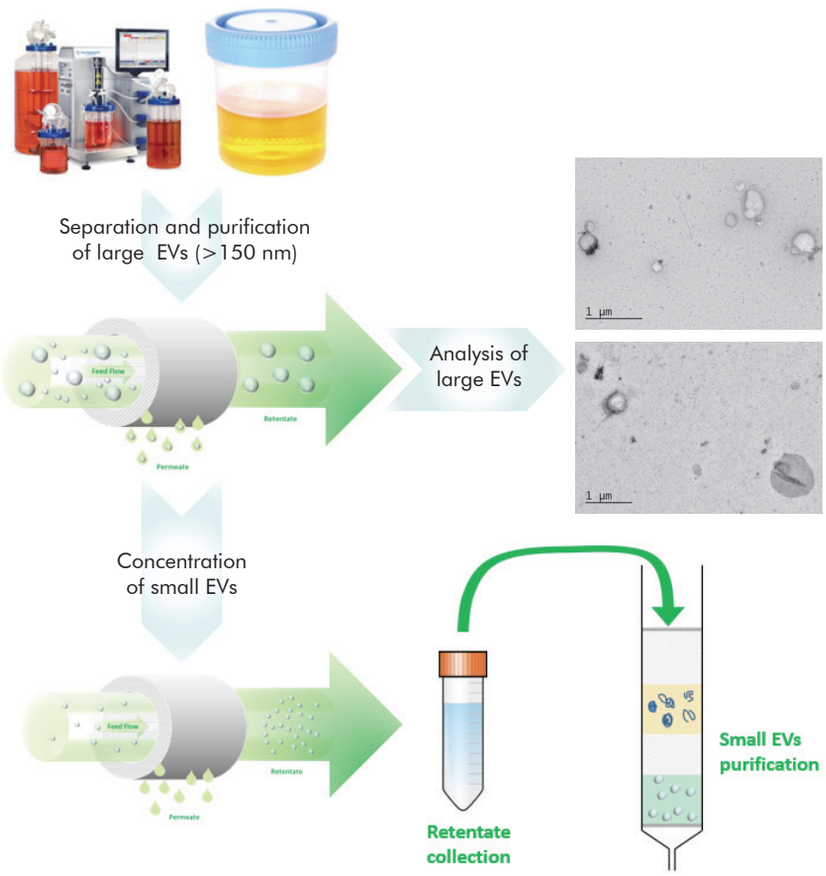
TFF-MV TFF-Easy Maxi-PURE-EVs

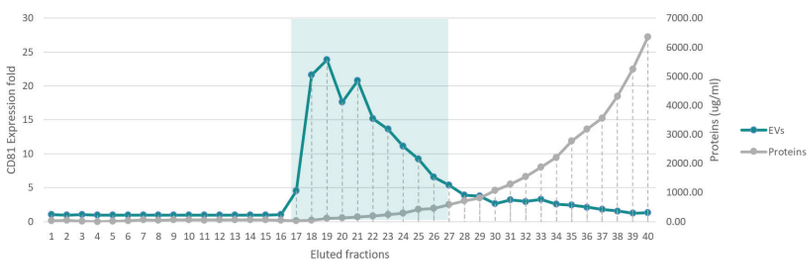
PURE-EVs PLUS and Maxi-PURE-EVs PLUS: Concentration and purification of EVs from diluted matrices.

TFF-Easy Maxi-PURE-EVs

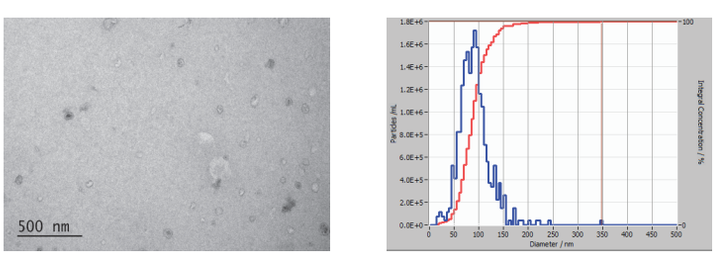
Cat. Code	Column	TFF-Filter
PURE-EVs COMPLETE: Complete EV purification and fractionation		
HBM-PEV-C	1 Maxi PURE-EVs	1 TFF-MV, 1 TFF-Easy
PURE-EVs PLUS:		
HBM-PEV-P5	5 PURE-EVs	1 TFF-Easy
HBM-mxPEV-P1	1 Maxi PURE-EVs	1 TFF-Easy

PURE-EVs COMPLETE: Scalable purification of small and large Extracellular Vesicles





7. Analysis of SEC fractions for detecting EV and protein content.



8. EM image and particle size distribution chart of small EVs purified by SEC (Maxi-PURE-EVs).

200 ml of cell conditioned medium from HCT116 cells have been filtered through the TFF-MV, in order to separate the large vesicles. The permeate containing the small EVs has been collected in a clean bottle (roughly 200 ml). The retentate containing EVs larger than 150 nm has been recovered, after washing, with a syringe, injecting 5 ml of PBS 1X. Large EVs have been visualized by TEM analsysis.

The collected permeate, containing small EVs, has been concentrated 10 folds by TFF-Easy (final volume 20 ml), then purified through Maxi-PURE-EVs column. Collected fractions were analyzed by ELISA assay, detecting CD81, by TEM and NTA using the Zetaview (Particle Metrix).

Extracellular Vesicle isolation tools

Available products

- Transparent or white immunoplates for colorimetric or luminometric assay.
- Magnetic or Latex Immunobeads.

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

Plates are coated with antibodies against common surface antigens present on total EV population.

Cat. Code	Immunoplate	Antibody	Recommended for
ELISA Immunoplate for CD9 Positive Extracellular Vesicle 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 Vesicle capture			
HBM-POC-CC/T1	Transparent	Anti Human CD63 Mouse Monoclonal	Cell conditioned media, Biofluids
HBM-POC-CC/W1	White		
Custommade ELISA Immunoplate			
ELISA 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.			

Immunoplates for enrichment of Tumor-derived EVs

Tumor tissues secrete a large quantity of EVs which act as strong regulators of the cancerogenesis process, shuttling their cargo molecules to receipient cells. TM9SF4 and EpCAM are two proteins known to be widely expressed in tumor tissues and in tumor-derived EVs, showing the ability to enrich tumor-derived EVs from human biofluids.

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). Its level is highly expressed in tumor tissues as well as in tumore derived EVs.
Ref: Lozupone F. et al. 2015

EpCAM: EpCAM is a transmembrane glycoprotein highly expressed in rapidly growing epithelial tumors. It seems to play an important role in localization of EVs in numerous physiological and pathological 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		

Applications

- Capture and enrichment of Tumor derived EV 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 tools

Applications

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

Storage conditions

Unopened: 2 years, stored at 4°C.

Opened: 6 month stored at 4°C.

Amount of material to use

It is recommended to load 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 Neural and Glial EVs

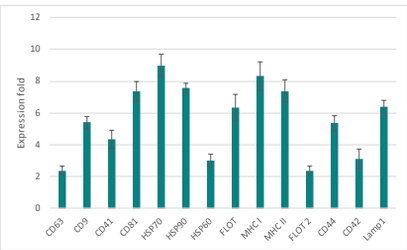
We developed Immunoplates coated with antibodies against EV surface antigens and indicative of neurological or glial origin.

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

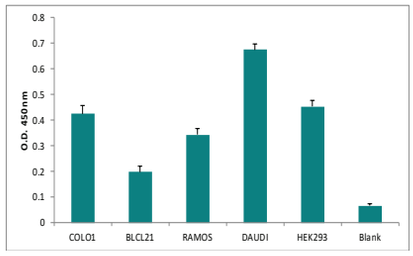
PLP1: PLP1is the major myelin protein from the central nervous system and plays an importan role in the formation and the maintenance of the myelin structure. EVs derived from glial cells are characterized by the presence of high levels of PLP1 protein.
Ref: Frühbeis et al. 2012

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

Immunoplates allow EV phenotyping without vesicle pre-purification steps



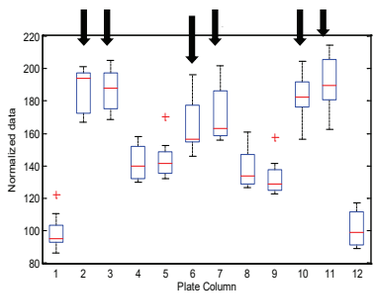
9. EV associated biomarkers analysis in a healthy donor's plasma sample.

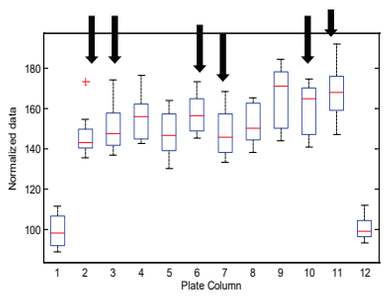


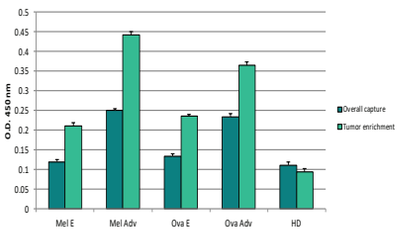
10. CD63 profiling of different cell derived EVs.

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

Enrichment of Tumor-derived Extracellular Vesicles



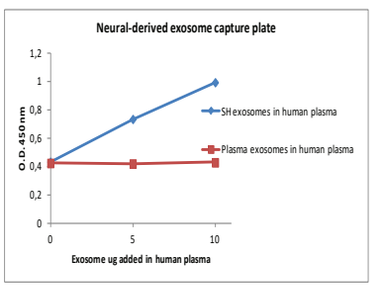




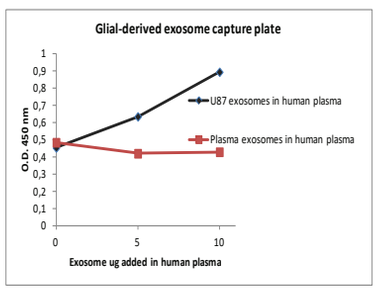
11. Enrichment in Tumor-derived EVs in early and late stage melanoma (Mel E; Mel ADV) and ovary carcinoma (Ova E, Ova ADV).

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).

Enrichment of Nural or Glial derived Extracellular Vesicles



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.

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).

Extracellular Vesicle isolation tools

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 exosome marker profiling.
- Nucleic acids extraction.
- EV elution from immunobeads.

Latex/Magnetic Immunobeads for Total EV isolation

Latex or Magnetic immunobeads are covalently coupled with antibodies against common EV surface antigens (CD9, CD63). They allow to capture 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 once more. Beads are sold in packages of 10 reactions and are available in 2 sizes (0.4 and 1 micron diameter).

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.

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, chosen from our antibody list or sent by customers. Magnetic or Latex beads are available.			

Storage condition

Immunobeads must be stored at 4°C.

Latex/Magnetic 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 EVs from human biofluids, thus providing a potential new platform for the research in circulating tumor biomarkers.

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
Custom Latex or Magnetic Immunobeads for enrichment of EV subpopulations			
Beads can be covalentely coated with EV binding antibodies against biomarker indicative of different origin. See service section, the antibodies can be chosen from our antibody list or sent by customers. Magnetic or Latex beads are available.			

Applications

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

Amount of material to use

Recommended starting volume from 0.1 ml to 0.5 ml of plasma, and from 0.5 ml to 1 ml of serum.

It is recommended to concentrate 10X urine and cell culture medium samples prior capture according to our suggested protocol (see page 19, TFF-Easy).

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 at least once more.

Custom Immunobeads

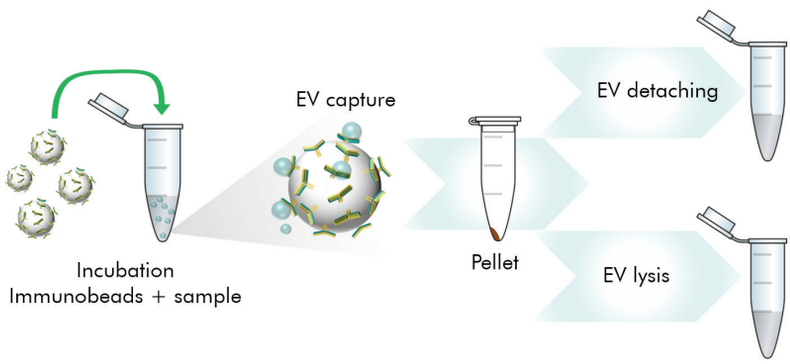
Latex immunobeads can be customized with antibodies chosen by customer. For information contact us at:

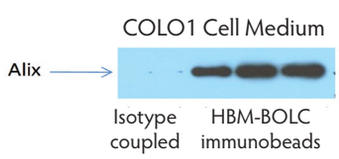
info@hansabiomed.eu

Extracellular Vesicle isolation tools

Immunobeads allow EV 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 two times more (fig 15).



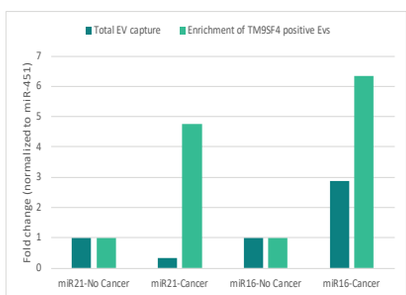


14. Alix expression by western blotting of exosomes captured on HBM-BOLC immunobeads from COLO1 cell supernatant vs isotype coupled beads.

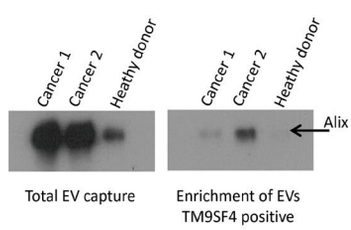
15. Western Blotting analysis of immunocaptured exosomes on beads.

TM9SF4 coated immunobeads enrich Tumor-derived EVs in cancer patients

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.



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

EXO-Prep: one step EV isolation reagent

EXO-Prep is a fast and convenient method for 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. EV pellet can be resuspended in buffered solutions. 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.

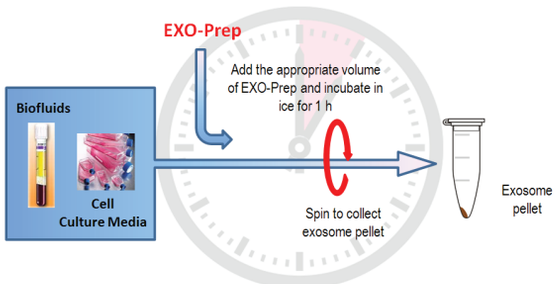
Applications

- 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).

Cat. Code	Volume	Reactions
EXO-Prep for EV Isolation from Plasma and Serum		
HBM-EXP-B5	5 ml	180 reactions with Plasma, 80 reactions with Serum
EXO-Prep for EV Isolation from Cell Media		
HBM-EXP-C25	25 ml	25 reactions
HBM-EXP-C50	50 ml	50 reactions
EXO-Prep for EV Isolation from Urine		
HBM-EXP-U25	30 ml	25 reactions
HBM-EXP-U50	60 ml	50 reactions

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).



References

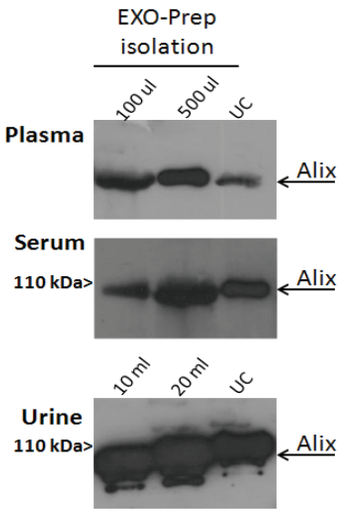
Wu, H. H., & Lee, O. K. (2017). Exosomes from mesenchymal stem cells induce the conversion of hepatocytes into progenitor oval cells. Stem Cell Research & Therapy, 8(1), 117.

Hovsepian, T., Saint-Auret, G., & Lebot, C. (2017). A Skin Extracellular Signaling Pathway via Intercellular Exosomes Highlighted by Genetic and Epigenetic Analysis Applied to a Natural Ingredient.

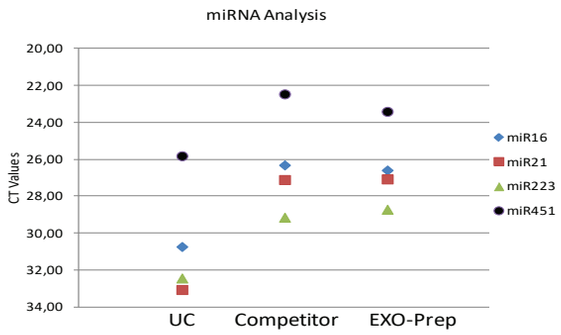
Extracellular Vesicle isolation tools

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

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).



18. WB analysis of Alix in EV lysates



19. Profiling of 4 miRNA EV associated.