2 Extracellular Vesicle Isolation Tools

Summary Section 2

2 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

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

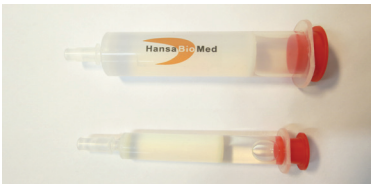
Distributors:
www.hansabiomed.eu/index.php/distributors

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 alterate 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.

PURE-EVs SEC Columns

New and efficient size exclusion chromatography (SEC) columns for the isolation of highly pure exosomes from biofluids (i.e. plasma, serum and urine) and from cell culture media.



TFF-Easy

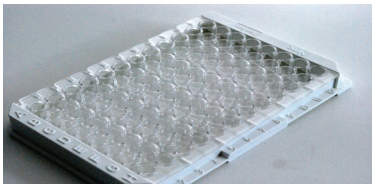
Hollow fiber cartridge which allows easily to concentrate cell conditioned media or diluted matrices by small scale tangential flow filtration (TFF), prior to EV isolation.

EXO-Prep

Easy one-step method for total exosome isolation from biofluids (i.e. plasma, serum and urine) and from cell culture media.

Immunoplates

For exosome immunocapture using generic or specific exosome-associated biomarkers (i.e. tumoral, neural and glial derived).



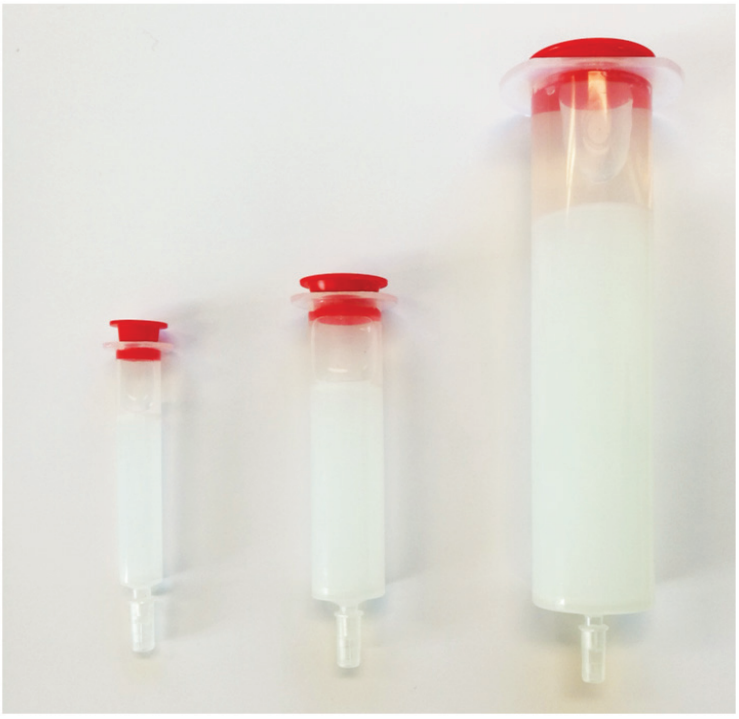
Immunobeads

Latex immunobeads for generic and specific exosome immunocapture from human biofluids or cell supernatants. Immunocaptured exosomes can be recovered and used for downstream analyses.

PURE-EVs Size Exclusion Chromatography Columns

Size Exclusion Chromatography (SEC) is considered one of the best methods for isolating and purifying exosomes and extracellular vesicles (EVs) from different matrices. In particular, this technique is very efficient for separating EVs from the circulating proteins and does not affect the original shape and functionality of the vesicles.
HBM-LS has developed different classes of SEC columns for EV purification from different volume and matrices.

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-10	1 ml - 20 ml	10 Columns
HBM-mxPEV-20	1 ml - 20 ml	20 Columns



miniPURE-EVs **PURE-EVs** **maxiPURE-EVs**

Applications

- Exosome and extracellular vesicles isolation from biofluids and cell media.
- Purification of pre-isolated EVs from contaminants.
- Isolated exosomes are suitable for multiple analyses (NTA, ELISA, FACS, WB, EM, MS, nucleic acid extraction, etc).

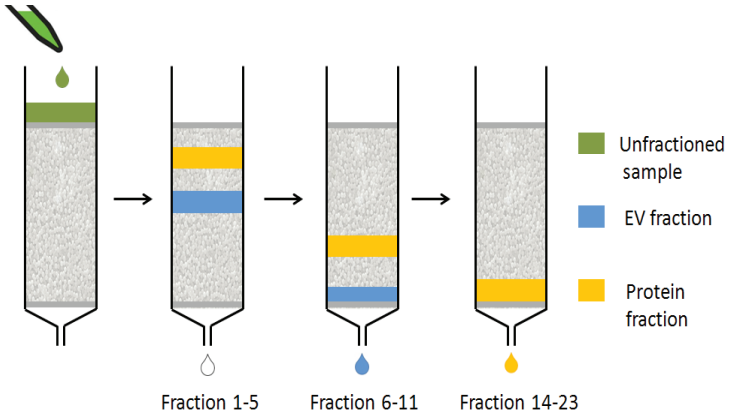
Advantages

- Easy and fast protocol (turnaround time approximately 15 minutes).
- Isolate exosomes from small sample volumes.
- Reusable up to 5 times.
- Easy to store and ship (4°C).

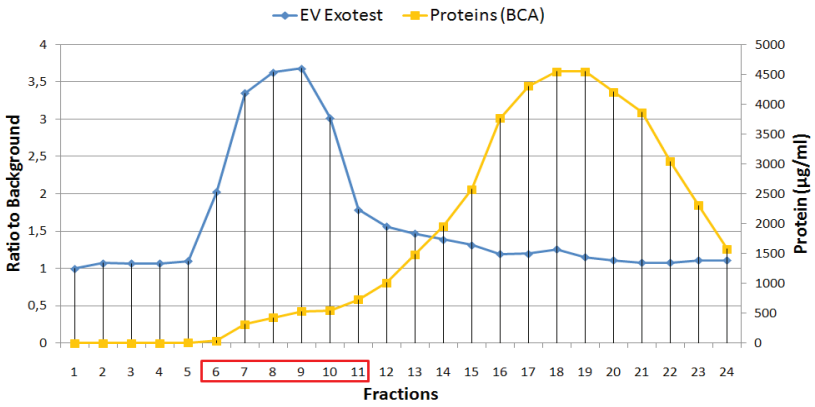
2 Extracellular Vesicle Isolation Tools

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

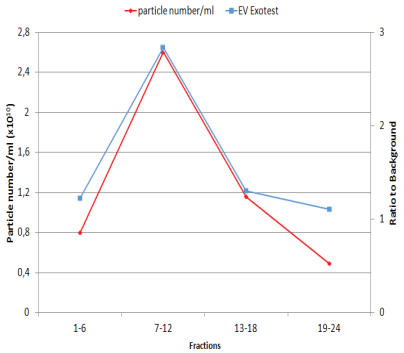
PURE-EVs column was rinsed with 1 ml of human plasma, 24 fractions (500 µl each one) have been collected and analyzed by ELISA ExoTEST™ assay (see section 3, ExoTEST quantification kit) and by BCA test for determining respectively the exosome and total protein content. As shown in figure 1 and 2, EVs are eluted in fractions 6 - 11 (turnaround time approximately 15 min), whereas the BCA analysis showed that the peak of plasma proteins corresponded to the fractions 14 - 24. The 24 fractions were collected in 4 groups (1-6; 7-12; 13-18; 19-24) and analyzed by NTA with Nanosight. Figures 3, 4 and 5 show the correlations between the eluted fractions and the performed analysis: positive matching between ExoTEST™ results and the particles analysis by NTA (fig 3), complete separation of EV from the plasma circulating proteins (fig 4 and 5)



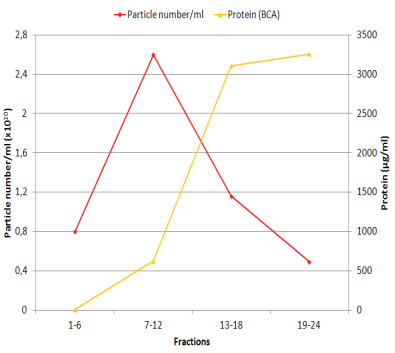
1. Exosome isolation from human plasma by PURE-EVs columns.



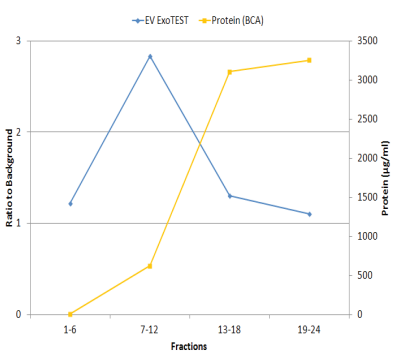
2. Matching of EV quantity and total protein content eluted in each single fraction. ExoTEST™ analysis shows that EVs are eluted in fractions 6-11 and successfully separated from the plasma circulating proteins (eluted in fractions 14-24). ExoTEST results expressed in ratio-to-background.



3. EV elution peak. ExoTEST™ vs NTA analysis



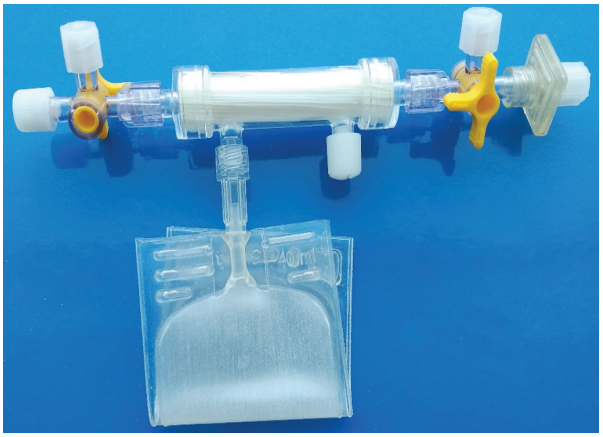
4. EV elution vs circulating protein elution. NTA analysis compared to protein BCA test



5. EV elution vs circulating protein elution. ExoTEST™ analysis compared to protein BCA test

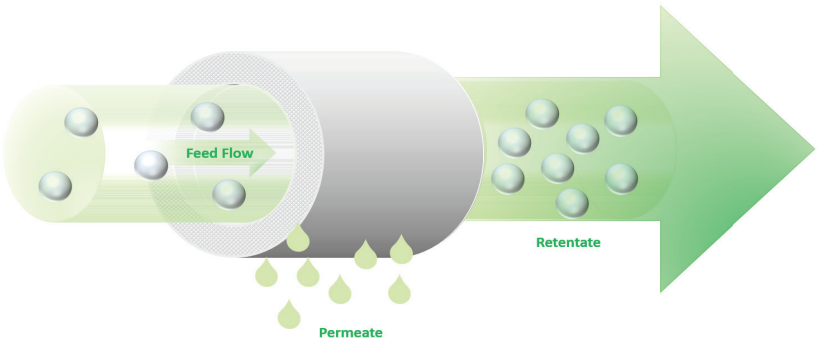
TFF-Easy: Tangential Flow Filter for EV concentration

Tangential flow filtration (TFF) is a rapid and efficient method, usually used for separation and purification of biomolecules. TFF can be also used to concentrate and desalt sample solutions, and is emerging as a new technique for EV isolation,if coupled with SEC.



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.



TFF-Easy can be easily washed and it is reusable multiple times.

Applications

- Concentration of diluted matrices 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.

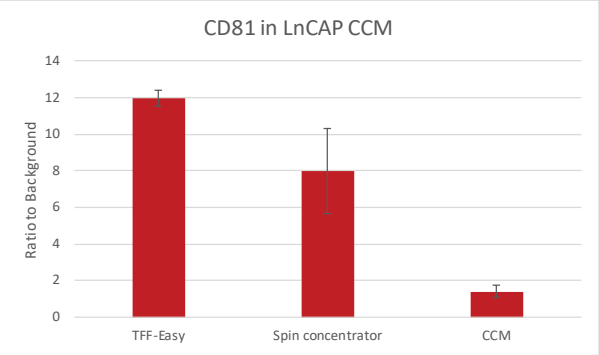
Advantage

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

2 Extracellular Vesicle Isolation Tools

TFF-Easy: Fast and efficient concentration of cell conditioned media or urine

LnCAP cell conditioned medium (CCM) has been concentrated in MWCO spin concentrator (100 kDa) and in TFF-Easy, alternatively. Expression of CD81 has been tested in ELISA assay. TFF-Easy showed better performance and low variability between different samples.



6. CD81 expression in concentrated CCM vs not concentrated (CCM).

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

TFF-Easy allows to change the buffer composition without the dialysis process. In the process described in figure 7 we tested the EV transfer from buffer 1 (PBS1x) to buffer 2 (deionized water*), which present different conductivity.
The TFF-Easy allows the complete removal of buffer 1, without affecting the EV concentration.

Buffer changing process	Conductivity (µS/cm)	Particle concentration (particle/ml)
EVs in buffer 1 (PBS1x)	15000	$4.3x10^{11}$
1- Buffer 1 (PBS1x) removal by TFF	15000	
2- EV dilution in buffer 2 (water)	240	
3- Buffer 1 (PBS1x) residue removal	55	
4- EV dilution in buffer 2 (water)	22	
EV in buffer 2 (water*)	12	$3.58x10^{11}$

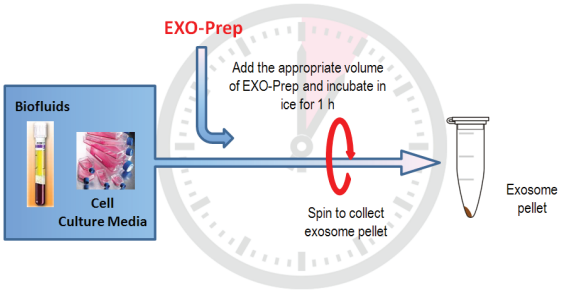
7. Process for changing EV buffer with TFF-Easy.

* EVs are not stable in deionized water. The water as buffer was chosen just to show the difference of conductivity between buffer 1 and buffer 2, and the complete removal of buffer 1.

EXO-Prep: one step EV isolation reagent

EXO-Prep is a fast and convenient method of Extracellular Vesicle isolation from biofluids and cell culture supernatants. 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 suitable for a wide range of analyses, such as NTA, protein profiling by using different techniques (western blotting, ELISA, FACS), nucleic acid extraction and profiling of mRNA or small RNA markers.

Cat. Code	Volume	Reactions
EXO-Prep for Exosome Isolation from Plasma and Serum		
HBM-EXP-B5	5 ml	180 reactions Plasma, 80 reactions Serum
HBM-EXP-B10	10 ml	350 reactions Plasma, 160 reactions Serum
EXO-Prep for Exosome Isolation from Cell Media		
HBM-EXP-C25	25 ml	25 reactions
HBM-EXP-C50	50 ml	50 reactions
EXO-Prep for Exosome Isolation from Urine		
HBM-EXP-U25	30 ml	25 reactions
HBM-EXP-U50	60 ml	50 reactions



Applications

- Single step isolation of EVs from biofluids and cell supernatants.
- Isolate the overall vesicles population in a sample.
- Isolated EVs can be used for NTA analysis.
- Isolated EVs are suitable for protein profiling (WB, ELISA, FACS).
- Isolated EVs are suitable for nucleic acid extraction and profiling.

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

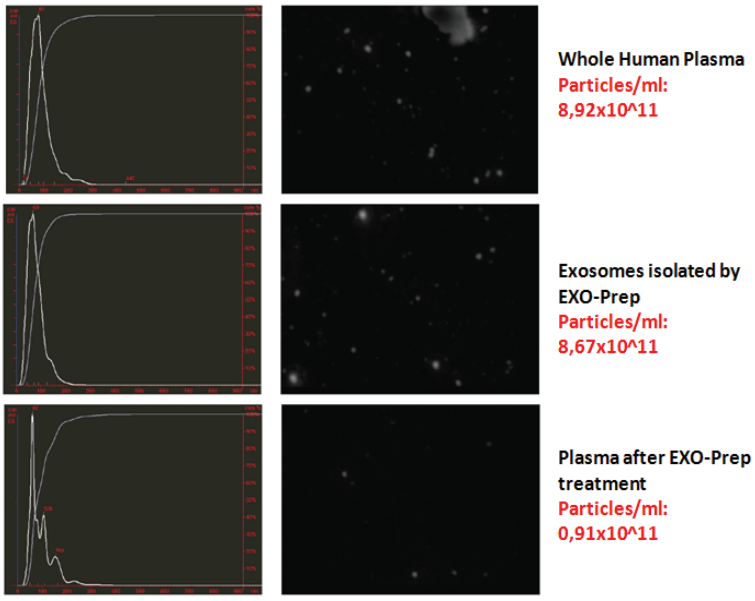
Wu, H. H., & Lee, O. K. (2017). Exosomes from mesenchymal stem cells induce the conversion of hepatocytes into progenitor oval cells. Stem Cell Research & Therapy, 8(1), 117.

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.

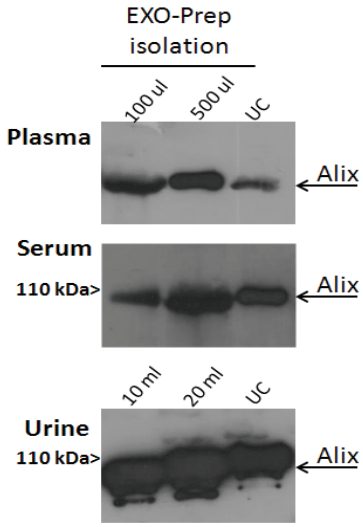
2 Extracellular Vesicle Isolation Tools

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

NTA analysis (Fig 8) of plasma sample pre- and post-EXO-Prep treatment shows isolation of almost the entire nanoparticle population. The number of nanoparticles isolated by EXO-Prep (8.67x10^11) closely corresponds to the estimated number present in whole plasma (8.92x10^11). Vice versa, the remaining plasma supernatant is almost completely depleted of nanoparticles (0.91x10^11). Isolated vesicles are suitable for multiple dowstream analyses, such as protein profiling via western blotting (Fig 9) or ELISA (Fig 10) or miRNA marker profiling (Fig 11).

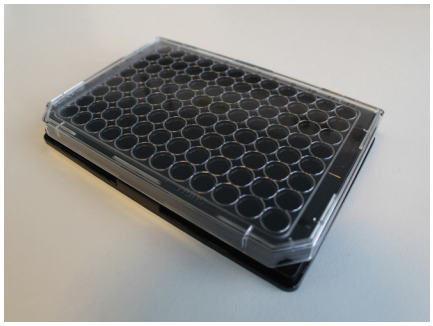


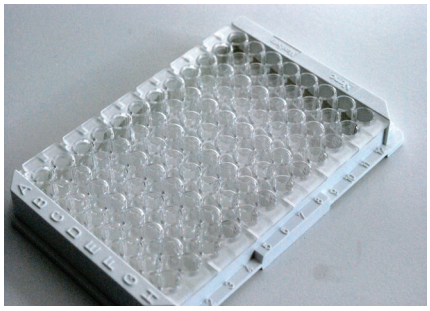
8. NTA and exosome detection in whole plasma (A), EXO-Prep isolated exosomes resuspended in 100 ul of PBS 1x (B) and plasma post-EXO-Prep treatment (C).



9. Detection of exosome marker Alix in protein lysates (30 µg) from exosomes isolated with EXO-Prep from plasma, serum and whole urine. Protein lysates (30 µg) from exosomes purified by ultracentrifugation (UC) were used as control.

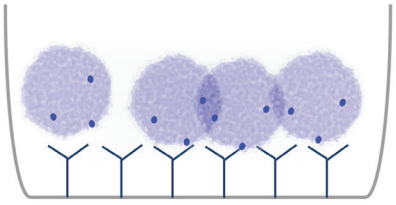
Immunoplates for Exosome capture and isolation





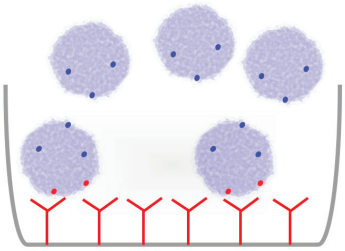
HBM-LS Immunoplates are 96 multi-well plates covalently pre-coated with specific exosome-binding antibodies allowing exosome capture and isolation from different sources (cell supernatant, human plasma, serum, urine and saliva). Covalent coating improves the correct orientation of antibodies maximizing the quantity of immuno-captured exosomes and increasing the binding efficiency of the plate. In addition the coating chemistry reduces the aspecific binding of circulating protein complexes and cell debris, and it helps the enrichment of exosome subpopulations. HBM-LS has developed different types of plates for capturing the overall or enriching specific exosome subpopulations (tumoral, neural, glial, monocytes and platelets). Plates are blocked and stabilized for long-term storage.

Immunoplates for capturing the overall Exosomes



Plates are coated with antibodies against exosome surface antigens present on overall exosome population.
- From human biofluids (plasma, serum, urine, saliva)
- From cell media of human or mouse cell lines

Immunoplates for enriching Exosome subpopulations



Plates are coated with antibodies overexpressed in particular pathological conditions on exosome surface.
- Capture from human plasma
- Capture and enrichment of tumor, glial, neuro, platelet derived exosomes

Applications

- Multiple profiling of exosomal markers from a single sample or screening of a large number of samples.
- Exosome capture and quantification from human biofluids (plasma, serum, urine, saliva).
- Exosome capture and quantification from human or mouse cell media.
- Titration of purified exosomes.
- Capture and enrichment of specific exosome subpopulations.

Advantages

- Ready to use.
- Long term storage (up to 2 years).
- No exosome pre-purification required (by ultracentrifugation or other methods).
- Small amount of sample required (100 µl per well).
- Suitable for nucleic acid extraction from exosome captured on the plate.
- Flexibility in designing a multiplexing assay.
- Open platform for customized coating solutions.

2 Extracellular Vesicle Isolation Tools

Immunoplate types

Different plates are available for colorimetric, luminometric or fluorimetric readings (transparent, white and black, respectively).

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 exosome 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.

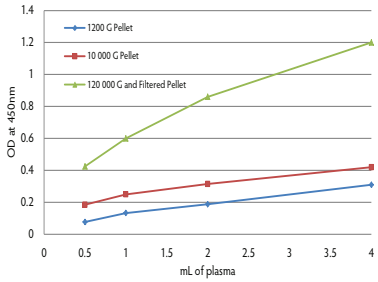
Immunoplates for capturing the overall Exosomes

Cat. Code	Type of immunoplate	Package size	Coating antibody
Overall Exosome capture from human plasma and urine			
HBM-POF-CC/T1 or T5	Transparent	1 or 5 plates	Rabbit
HBM-POF-CC/W1 or W5	White	1 or 5 plates	Rabbit
HBM-POF-CC/B1 or B5	Black	1 or 5 plates	Rabbit
Overall Exosome capture from human serum			
HBM-POS-CC/T1 or T5	Transparent	1 or 5 plates	Mouse
HBM-POS-CC/W1 or W5	White	1 or 5 plates	Mouse
HBM-POS-CC/B1 or B5	Black	1 or 5 plates	Mouse
Overall Exosome capture from human saliva			
HBM-POSL-CC/T1 or T5	Transparent	1 or 5 plates	Rabbit
HBM-POSL-CC/W1 or W5	White	1 or 5 plates	Rabbit
HBM-POSL-CC/B1 or B5	Black	1 or 5 plates	Rabbit
Overall Exosome capture from cell media			
HBM-POC-CC/T1 or T5	Transparent	1 or 5 plates	Mouse
HBM-POC-CC/W1 or W5	White	1 or 5 plates	Mouse
HBM-POC-CC/B1 or B5	Black	1 or 5 plates	Mouse

Immunoplates for enriching Exosome subpopulations

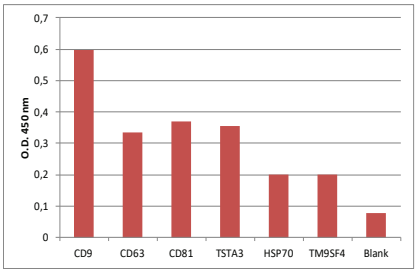
Cat. Code	Type of immunoplate	Package size	Coating antibody
Tumor-derived Exosome capture and enrichment from human plasma			
HBM-PTF-CC/T1 or T5	Transparent	1 or 5 plates	Rabbit
HBM-PTF-CC/W1 or W5	White	1 or 5 plates	Rabbit
HBM-PTF-CC/B1 or B5	Black	1 or 5 plates	Rabbit
Neural-derived Exosome capture and enrichment from human plasma			
HBM-PNF-CC/T1 or T5	Transparent	1 or 5 plates	Rabbit
HBM-PNF-CC/W1 or W5	White	1 or 5 plates	Rabbit
HBM-PNF-CC/B1 or B5	Black	1 or 5 plates	Rabbit
Glial-derived Exosome capture and enrichment from human plasma			
HBM-PGF-CC/T1 or T5	Transparent	1 or 5 plates	Mouse
HBM-PGF-CC/W1 or W5	White	1 or 5 plates	Mouse
HBM-PGF-CC/B1 or B5	Black	1 or 5 plates	Mouse

Immunoplates allow exosome protein profiling without vesicle pre-purification steps

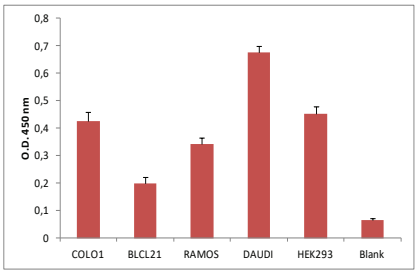


12. Selectivity in capturing purified exosomes (pellet at 120000g) and no other circulating microvesicles (pellet 1200g and 10000g)

HBM-LS plates are useful tools for immunocapturing exosomes from biofluids or culture media, for protein analyses and protein marker profiling. No significant cross-reactivity is observed with soluble antigens or other vesicle-associated proteins (Fig 12). They allow quantitative and qualitative analysis of different protein markers (Fig 13) from the same sample, or expression profiling of a single marker in different samples (Fig 14), without exosome pre-purification via ultracentrifuge or other methods.

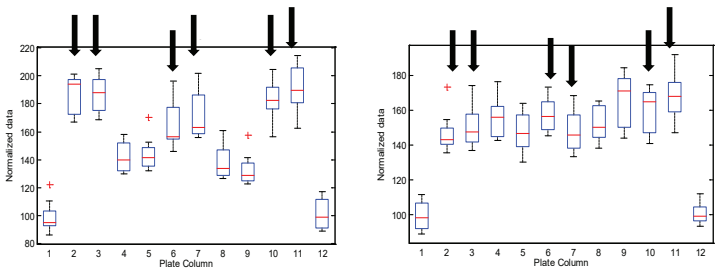


13. Common exosomal biomarkers analysis in a healthy donor's plasma sample



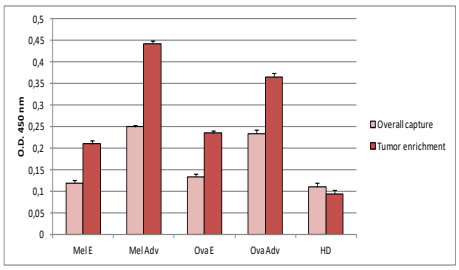
14. CD63 profiling on exosomes derived from supernatants of different cell lines

Immunoplates allow exosome protein profiling without vesicle pre-purification steps

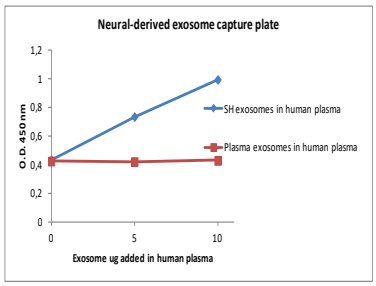


15. Immunoplate for tumor-derived exosomes capture (HBM-PTF) allows discrimination of cancer patients (black arrows) from controls, not detectable using Overall Exosome capture (HBM-POF).

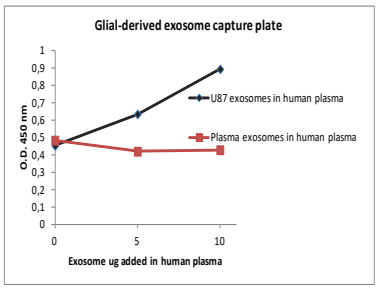
Pre-coated plates with proprietary capturing antibodies directed to antigens indicative of pathological states (tumoral, neural, glial) and enable isolation and enrichment of exosomes subpopulations. All immunoplates, showing similar high specificity and low background of the Overall Exosome capture plates, are a unique tool for studying specific exosome subpopulations.



16. Enrichment in Tumor-derived exosome in early and late stage melanoma (Mel E; Mel ADV) and ovary carcinoma (Ova E, Ova ADV) using Tumor-derived exosome capture plate vs Overall exosome capture plate.



17. Specific immunocapture of neuroblastoma (SH) derived exosomes spiked in human plasma from healthy donors using HBM-PNF



18. Specific immunocapture of glioblastoma (U87) derived exosomes spiked in human plasma from healthy donors using HBM-PGF

2 Extracellular Vesicle Isolation Tools

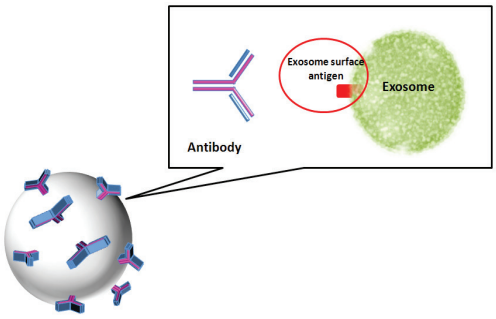
Applications

- Overall exosome isolation from cell culture media and human biofluids (tested for plasma, serum, urine).
- Overall exosome isolation from mouse biofluids (tested for plasma and serum).
- Capture and enrichment of human exosome subpopulation (tumor-derived).
- Downstream exosome marker profiling.
- Nucleic acids extraction
- Exosome elution from immunobeads

Advantages

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

Immunobeads for Exosome capture and isolation



HBM-LS provides several types of Immunobeads for capturing and isolating overall or specific exosome sub-populations. Latex immunobeads are covalently coupled with antibodies against exosome surface antigens, allowing exosome capture from human biofluids (tested for plasma, serum and urine) and cell culture media without pre-purification steps (ultracentrifuge or other method for exosome purification). HBM-LS immunobeads are able to capture the overall exosome population (Immunobeads for Overall Exosome capture) or to enrich exosome subpopulation derived from tumoral source (Tumor-derived exosome capture and enrichment).

References

Zarovni, N., Corrado, A., Guazzi, P., Zocco, D., Lari, E., Radano, G., ... & Chiesi, A. (2015). Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches.Methods.

Jia, S., Zocco, D., Samuels, M. L., Chou, M. F., Chammas, R., Skog, J., ... & Kuo, W. P. (2014). Emerging technologies in extracellular vesicle-based molecular diagnostics. Expert review of molecular diagnostics, 14(3), 307-321.

Latex or magnetic Immunobeads for Exosome capture

All Immunobeads are available in two sizes (0.4 and 1 micron of diameter) and are sold in packages of 10 and 20 reactions, or as TEST format that includes material for 3 and 5 reactions. Immunobeads are supplied with an Exosome Elution Buffer that allows detachment and elution of captured exosomes for downstream analyses, and with a Beads Regeneration Buffer to regenerate immunobeads for further usage.

Cat. Code	Immunobead diameter	Package	Coating antibody
Overall Exosome immunocapture from human biofluids			
HBM-BOLF-CC/10-04	0.4 micron	10 reactions	Mouse
HBM-BOLF-CC/10-1	1 micron	20 reactions	Mouse
HBM-BOLF-CC/20-04	0.4 micron	10 reactions	Mouse
HBM-BOLF-CC/20-1	1 micron	20 reactions	Mouse
Immunobeads are also available in TEST format for 3 and 5 reactions. Cat Code: HBM-TBOLF-CC/# (3 or 5 reactions)-## (bead diameter, 0.4 µm or 1 µm)			
Overall Exosome immunocapture from cell culture media			
HBM-BOLC-CC/10-04	0.4 micron	10 reactions	Mouse
HBM-BOLC-CC/10-1	1 micron	20 reactions	Mouse
HBM-BOLC-CC/20-04	0.4 micron	10 reactions	Mouse
HBM-BOLC-CC/20-1	1 micron	20 reactions	Mouse
Immunobeads are also available in TEST format for 3 and 5 reactions. Cat Code: HBM-TBOLC-CC/# (3 or 5 reactions)-## (bead diameter, 0.4 µm or 1 µm)			
Tumor-derived Exosome immunocapture from human biofluids			
HBM-BTLF-CC/10-04	0.4 micron	10 reactions	Rabbit
HBM-BTLF-CC/10-1	1 micron	20 reactions	Rabbit
HBM-BTLF-CC/20-04	0.4 micron	10 reactions	Rabbit
HBM-BTLF-CC/20-1	1 micron	20 reactions	Rabbit
Immunobeads are also available in TEST format for 3 and 5 reactions. Cat Code: HBM-TBTLF-CC/# (3 or 5 reactions)-## (bead diameter, 0.4 µm or 1 µm)			
Overall Exosome immunocapture from mouse plasma and serum			
HBM-BMLF-CC/10-04	0.4 micron	10 reactions	Mouse
HBM-BMLF-CC/10-1	1 micron	20 reactions	Mouse
HBM-BMLF-CC/20-04	0.4 micron	10 reactions	Mouse
HBM-BMLF-CC/20-1	1 micron	20 reactions	Mouse
Immunobeads are also available in TEST format for 3 and 5 reactions. Cat Code: HBM-TBMLF-CC/# (3 or 5 reactions)-## (bead diameter, 0.4 µm or 1 µm)			
Overall Exosome immunocapture from mouse cell media			
HBM-BMLC-CC/10-04	0.4 micron	10 reactions	Mouse
HBM-BMLC-CC/10-1	1 micron	20 reactions	Mouse
HBM-BMLC-CC/20-04	0.4 micron	10 reactions	Mouse
HBM-BMLC-CC/20-1	1 micron	20 reactions	Mouse
Immunobeads are also available in TEST format for 3 and 5 reactions. Cat Code: HBM-TBMLC-CC/# (3 or 5 reactions)-## (bead diameter, 0.4 µm or 1 µm) .			

Storage condition

Immunobeads must be stored at 4°C.

Amount of material to use

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).

Packaging information

Immunobeads (10 and 20 reaction packages) 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

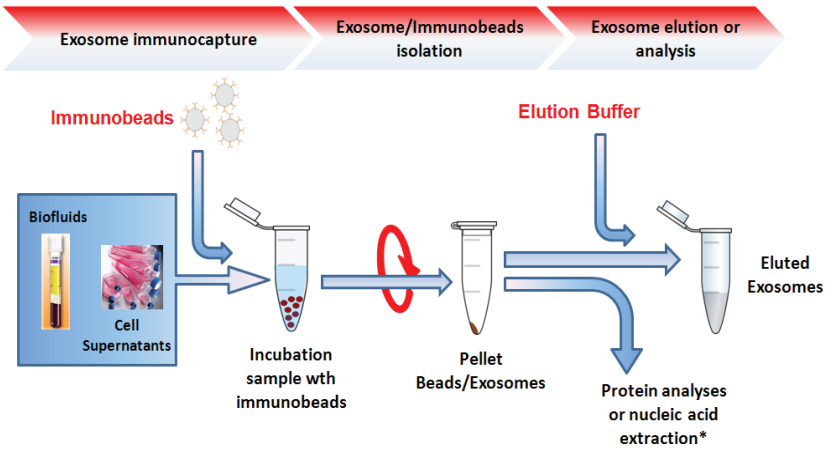
Latex immunobeads can be customized with antibodies chosen by the customer. For information contact us at:

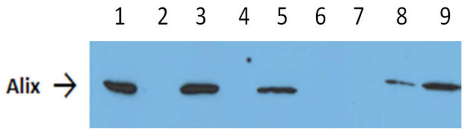
info@hansabiomed.eu

2 Extracellular Vesicle Isolation Tools

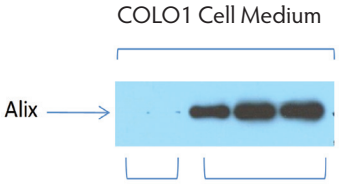
Immunobeads allow exosome capture and multiple downstraem analyses

Following incubation, beads can be recovered by centrifugation, resuspended in Laemmli buffer for SDS-PAGE and western blotting analysis (Fig 19, 20) or in appropriate lysis buffer for nucleic acid analysis (fig 24). Alternatively the vesicles can be eluted from the beads with the Elution Buffer and used for downstream applications such as ELISA (Fig 21), EM, etc. Eluted beads can be regenerated with Bead Regeneration Buffer and reused for capturing exosomes two times more (Fig 22).

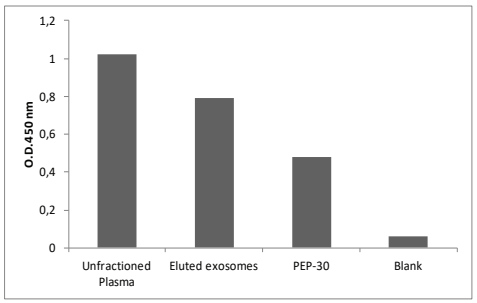




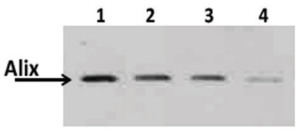
19. Alix expression by western blotting of human plasma exosomes captured on HBM-LS Immunobeads from 0.5 ml of plasma in comparison with exosomes purified via ultracentrifuge. 1, 3 and 5: Immunocapture with immunobeads from 1 ml, 0.5 ml and 100 µl of human plasma respectively. 2, 4, 6: Ultracentrifuged exosomes from plasma after immunobeads capture. 7 Immunocapture with immunobeads isotype coupled. 8, 9: Ultracentifuged exosome from 0.5 ml and 1 ml of human plasma.



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

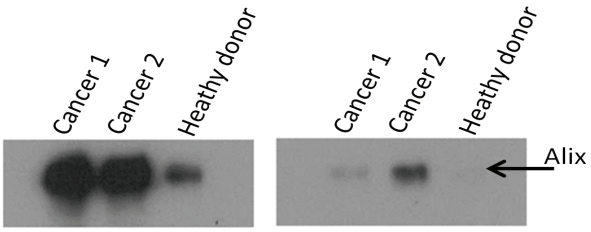


21. CD9 expression in exosomes eluted from immunobeads



22. Western Blotting analysis of immunocaptured exosomes on beads.
1- Exosomes immunocaptured with fresh beads
2- Exosomes immunocaptured with beads reused once
3- Exosomes immunocaptured with beads reused twice
4- Exosomes immunocaptured with beads reused the third time

Immunobeads enrich Tumor-derived Exosome subpopulation in cancer patient

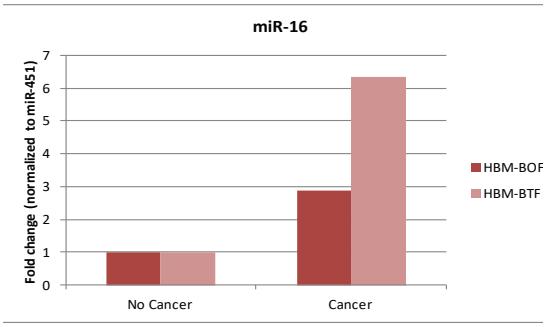


Immunobeads for Overall Exosome capture

Immunobeads for Tumor derived exosome capture

23. Anti-Alix WB analysis on exosomes immunocaptured with beads for overall (HBM-BOLF) and for tumor-derived exosomes (HBM-BTLF). WB shows the capture of exosomes only from cancer patients when beads for tumor-derived exosomes are used.

Immunobeads for tumor-derived exosome capture can be used for enriching exosome subpopulations derived from tumoral sources thus providing a novel platform for cancer biomarker research.



24. Enrichment in miR-16 expression level in cancer when immunobeads for Tumor-derived exosome capture are used. miR-16 expression was meeasured relative to control miR-451 by qPCR.

2 Extracellular Vesicle Isolation Tools