Chapter 3

Extracellular Vesicle characterization

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Extracellular Vesicle characterization

Introduction

EVs research needs of reliable, affordable and optimized tools for quantification and characterization of vesicles in complex biological samples as well as in cellular models. HBM-LS offers the ExoTEST™ and the Exo-FACS kits for quantification and characterization of EV markers by ELISA and FACS, respectively, and the new Enolase Activity Kit, which allows to characterize the different EVs sources by the determination of the enolase activity.

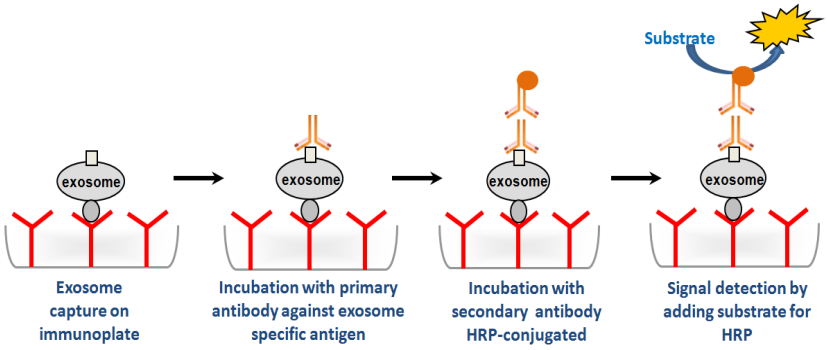
ExoTEST™: ELISA ready-to-use quantification kit

Exo-FACS: EV marker analysis by FACS

EV Enolase: Enolase activity on EVs

ExoTEST™: ELISA ready-to-use quantification kit

ExoTEST™ is a patented double sandwich ELISA assay for quantitative and qualitative analysis of vesicles. In particular, ExoTEST™ is a successful platform for exosome quantification and characterization from small amount of human biological fluids or cell conditioned media. ExoTEST™ enables reliable and precise quantitative measurement and comparison among samples and individual experiments and provides increased sensitivity in detection of EV markers with respect to other analytical methods (i.e FACS, WB).



ExoTEST™ consists of ELISA plates pre-coated with proprietary pan-exosome antibodies enabling specific capture of exosomes from different biological samples. Quantification and characterization of exosomal proteins is subsequently performed using appropriate detection antibodies against exosome surface antigens. Lyophilized Exosome Standards, characterized for protein content and particle number (NTA) allow the quantification of unknown sample by a standard calibration curve.

Kit components	Description
Immunoplate	96 well (12 strips x 8 wells) precoated with specific exosome capturing antibody.
Lyophilized Exosome Standards	Exosome Standards from human plasma, serum, urine, saliva or cell medium for calibration curve.
Antibodies for exosome marker detection	Primary anti-human CD9 antibody (HBM proprietary) and secondary antibody HRP conjugated for exosome detection.
Buffers	Sample buffer for antibody dilution and incubation. Washing buffer for washing ELISA plate.
Reagents	Reagents for signal detection.

Characteristics

- Starting material: 100 µl of biological sample. Whole plasma and serum can be directly loaded on the plate. Concentrate 10 folds urine or cell media prior plate loading.
- The detection limit of the assay is lower than 0.35 µg of EVs.
- Kit contains Lyophilized EVs for assay calibration.

Applications

- Exosome capture and quantification from human biofluids and cell culture media.
- Comprehensive exosome profiling.
- Pre-clinical research on non-invasive biomarkers for detection and monitoring of a number of pathological conditions (inflammation, cancer, neurodegeneration, etc).

Advantages

- Ready to use.
- No initial exosome purification required.
- User friendly and suitable for multiple marker analyses.
- Available in TEST format (limited to 3 ELISA strips, 24 wells).

Extracellular Vesicle characterization

ExoTEST™: ELISA ready-to-use quantification kit

HBM-LS offers different types of ExoTEST™ kits for quantification of Extracellular Vesicle population from human biofluids (plasma, urine, serum) and from cell culture supernatants. Furthermore, ExoTEST™ it is available for Tumor-derived EVs Enrichment and quantification.

Cat. Code	Description	Readout
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from human plasma and urine		
HBM-RTK-POF/##	EV detection performed with anti-human CD9 antibody and anti-mouse HRP conjugated.	Colorimetric
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from human serum		
HBM-RTK-POS/##	EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP.	Colorimetric
ExoTEST™ for Extracellular Vesicle immunocapture and quantification from cell culture media		
HBM-RTK-POC/##	EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP.	Colorimetric
ExoTEST™ for Tumor-derived Extracellular Vesicle immunocapture and quantification from human plasma		
HBM-RTK-PTF/##	EV detection performed with anti-human CD9 antibody and anti-mouse HRP conjugated.	Colorimetric
Custom made ExoTEST™ for Specific Extracellular Vesicle immunocapture and quantification		
HBM-RTK-CMK	HansaBioMed Life Sciences offers the flexibility of creating and designing your own kit by choosing among a wide variety of reagents available in our catalog. For information contact info@hansabiomed.eu	
Storage condition		
All reagents are shipped with ice packs and can be stored at 4-8° C.		
All kits are also available in TEST format, limited to 3 ELISA strips (24 wells). Cat Code: HBM-TRTK-###		

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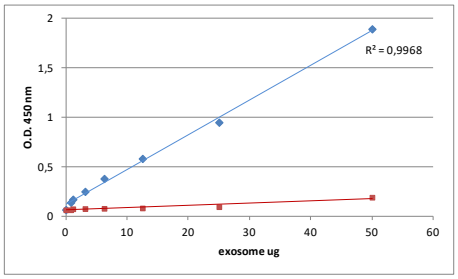
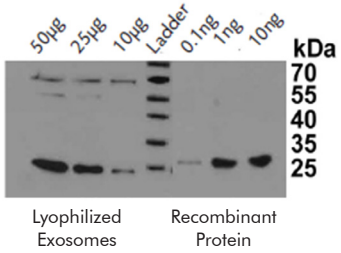
ExoTEST™: High sensitivity in detecting low exosome amount



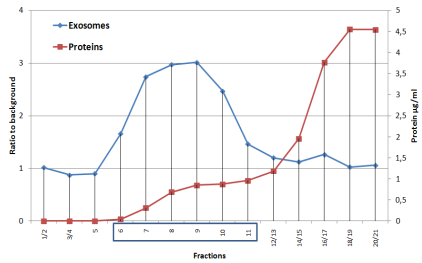
1. CD9 titration (blue line) of healthy donor plasma exosome standards (HBM-PEP100) and comparison with observed background (red line, only secondary antibody).



2. CD9 exosome marker detection by Western Blotting on lyophilized exosomes from human plasma (HBM-PEP100) and recombinant CD9 protein.

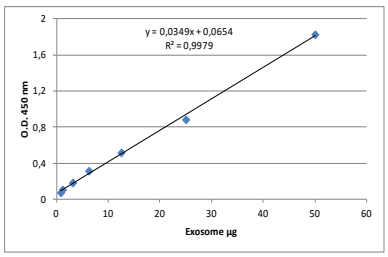
The sensitivity of the ExoTEST™ is higher than western blotting (Figures 1 and 2). 10 µg of lyophilized exosomes are equivalent to 0.1 ng of recombinant exosomal protein: since the standard curve's lower concentration is 0.39 µg of lyophilized exosomes (Fig 1), the sensitivity of our test is around 39 pg of protein equivalent.

ExoTEST™: Highly specific EV binding, without circulating protein contamination

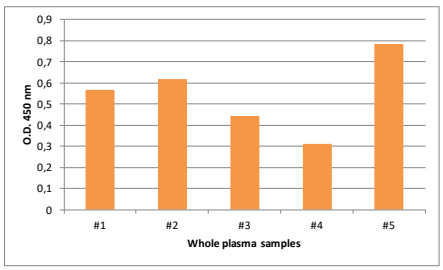


3. Comparison of ExoTEST™ analysis and total protein content (BCA test) of different fractions of human plasma fractioned by SEC column. EVs are eluted in fractions 6-11, whereas the highest yield of plasma proteins appears from fraction 14.

ExoTEST™: Example of Ev quantification from 5



4. Standard curve obtained with Lyophilized Exosome Standards from human plasma healthy donors (HBM-PEP100) with anti-CD9 antibody.



5. CD9 titration of exosomes in 5 different whole plasma from healthy donor.

Example of exosome quantification performed in 5 unknown plasma samples from healthy donors using the ExoTEST™ (HBM-RTK-POF/TP). Following the binding of Lyophilized Standards and unknown samples onto the ELISA plate, test is run according to the kit protocol and exosome detection is performed with anti-CD9 antibody (HBM-LS).
Exosome quantification is finally performed calculating the quantity of exosomes (expressed in µg) in the 5 unknown samples using the equation obtained from the standard curve (Fig 3). The particle number contained in 100 µl of plasma is calculated from quantity of exosomes (expressed in µg) according to the particle concentration (number of particles/ml) indicated in the label of the Lyophilized Exosome Standards (HBM-PEP100, NTA: 3x10^11 particles/ml).

Plasma sample	O.D. 450 nm	EV µg	Particle number in 100 µl of plasma
#1	0,5673	12,869	3,86x10^9
#2	0,6194	14,205	4,26x10^9
#3	0,4425	9,6692	2,90x10^9
#4	0,3100	6,2717	1,88x10^9
#5	0,7853	18,458	5,54x10^9

Extracellular Vesicle characterization

Applications

- Exosome isolation and exosome marker characterization via FACS.
- Comprehensive exosome profiling.

Advantages

- Ready to use.
- No initial exosome purification required.
- Lyophilized Exosome Standards for positive control included.
- User-friendly and suitable for multiple marker analyses.

Kit components

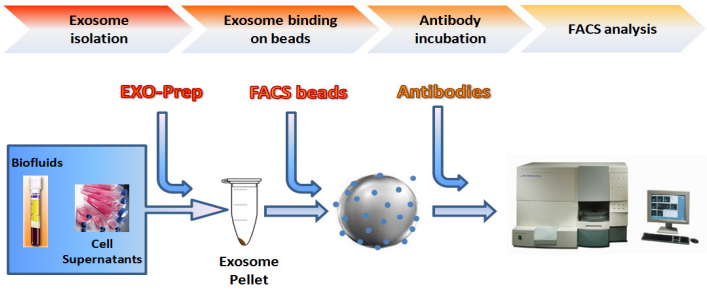
- EXO-Prep for exosome isolation.
- Lyophilized Exosome Standards as positive control.
- Primary antibody for exosome marker detection as positive control.
- Secondary antibody Alexa 488.
- Sample buffer, for antibody incubation.

Storage

All reagents are shipped and must be stored at 4°C.

Exo-FACS: ready-to-use kit for EV FACS analysis

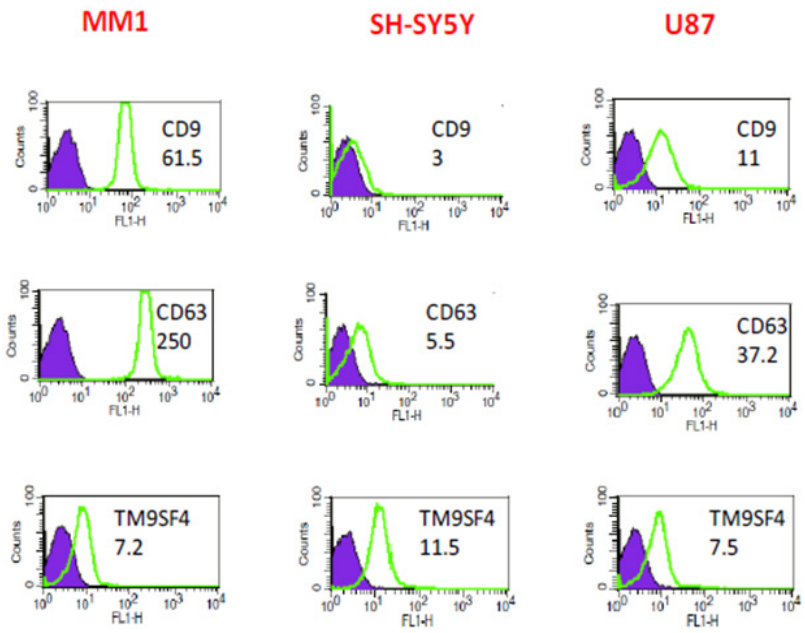
The kit consists of EXO-Prep reagent for exosome isolation, 4 µm beads used for the overall capture of pre-isolated exosomes, lyophilized exosomes from cell culture supernatants or human biological fluids as positive control. The characterization of exosomal proteins (membrane-expressed or internal) is subsequently performed using appropriate detection antibodies against exosome associated antigens.



HBM-LS offers different Exo-FACS kits for staining of EV markers from human biofluids (plasma, urine, serum, saliva) and from cell culture supernatants. Exo-FACS contains reagents for 20 reactions (lyophilized exosomes, beads, antibodies and buffers). Primary antibody included in the kit is against a common exosomal marker (CD9 or CD63) and can be used as a positive control for protein profiling via FACS analysis.

Cat. Code	Description	Lyophilized EV	Detection antibody
Exo-FACS ready to use kits for analysis of exosome marker from human biofluids			
HBM-FACS-PEP	FACS analysis of plasma EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PES	FACS analysis of serum EVs	HBM-PES100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PEU	FACS analysis of urine EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
HBM-FACS-PESL	FACS analysis of saliva EVs	HBM-PEP100 1 vial, 100 µg	Anti human CD9
Exo-FACS ready to use kits for analysis of exosome marker from cell culture media			
HBM-FACS-C	FACS analysis of cell derived EVs	HBM-###100 * 1 vial, 100 µg	Anti human CD63
* Possibility to choose the lyophilized EVs from the list of Lyophilized EVs from cell media available in the section 1 of this catalog, page 5 and 6.			

Exo-FACS: Exosome protein profiling by Flow Cytometry technique



6. FACS profiling of exosomal markers CD9, CD63 and TM9SF4 in purified exosomes from MM1, SH-SY5Y and U87 cell lines.

Exo-FACS was used for a protein marker profile in exosomes derived from different sources. Exosome binding on FACS-beads was performed by incubation at 4°C overnight. Exosome-bead complex is ready to be labeled with fluorophore-conjugated antibodies for specific exosome markers. In figure 6 is shown a profile of expression of three different exosome markers in exosomes purified from Melanoma (MM1), Neuroblastoma (SH) and Glioblastoma (U87) cell supernatants.

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Extracellular Vesicle characterization

Characteristics

- Starting material: minimum 1x10^8 particles per reaction.
- Kit contains Lyophilized EVs as positive control.
- Fluorimetric and colorimetric readout.

Applications

- EV characterization by funcional properties.
- Determination of the Enolase activity in purified/isolated EVs.
- Determination of the functionality and stability of EVs from cell lines.
- Mechanistic studies of EVs of cancer origin

Advantages

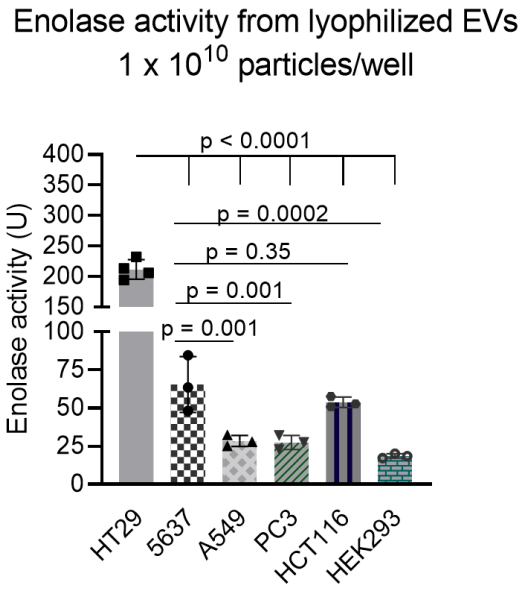
- Ready to use.
- Suitable for measuring enolase activity from fresh, frozen or lyophilized cell-derived EVs

EV-Enolase: Enolase activity on EVs

Enolase (EC 4.2.1.11), also called 2-phospho-D-glycerate hydrolase or 2-phosphoglycerate dehydratase, is a key enzyme in glycolysis. It converts 2-phosphoglycerate to phosphoenolpyruvate (PEP) & also catalyzes the reverse reaction, PEP to 2-phosphoglycerate under anabolic conditions during gluconeogenesis. This enzyme exists in all organisms, which can undergo glycolysis. Enolase activity is easily detectable in extracellular vesicles (EVs) derived from eukaryotic cells and it could be used for evaluating functionality and stability of EVs. Moreover, it's increased activity is associated with tumorigenesis and therefore precise measurement of enolase activity may be of great interest for EV-based tumor diagnosis.

Cat. Code	Description
HBM-K691-EN	Enolase activity on Extracellular Vesicles
Shippment and storage: Kit is shipped at controlled temperature with ice pack. Store the components as indicated in the product datasheet.	

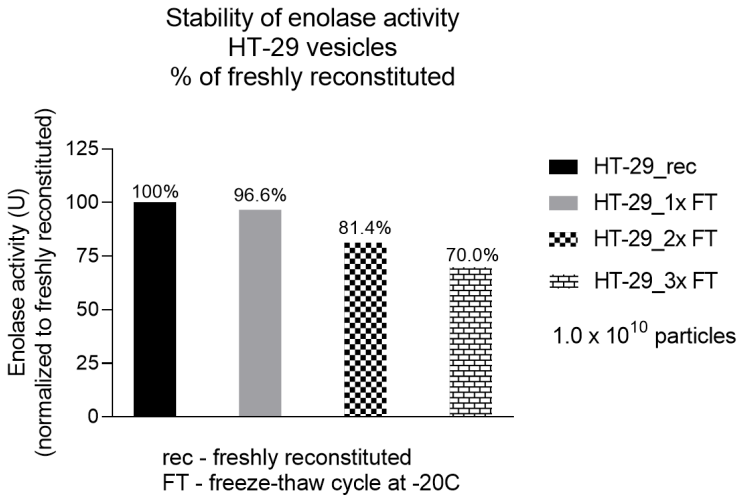
Cancer cell derived EVs show different profile of enolase activity



Enolase activity in EVs isolated from conditioned media of various cell lines. $1x10^{10}$ particles were used and the enolase activity was calculated based on the standard curve. Statistical analyses with one-way ANOVA and Dunnett's multiple comparison test. Symbols are biological repeats, bars indicate means and error-bars are SDs.

7. Enolase activity in EVs purified by SEC from different cancer cells.

Enolase activity is indicative of the EV state



Lyophilized HT-29 EVs were reconstituted in MilliQ water and 1, 2 or 3 freeze-thaw cycles at -20° C were performed. Enolase activity was normalized relative to the freshly reconstituted EVs (rec). One freeze-thaw cycle does not affect the enolase activity compared to the freshly reconstituted sample.

8. Stability of enolase activity in extracellular vesicles (EVs) after lyophilization and freezing/thawing cycles.