

## Highly Pure Lyophilized Exosomes

Lyophilization is an ideal technique for maintaining the stability of exosomes for long-term storage at 4°C. HansaBioMed provides lyophilized exosomes from human plasma, serum, urine and saliva or supernatants of a wide-range of cell lines.

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions, in a dynamic, regulated and functionally relevant manner. Both quantity and molecular composition of released exosomes depend on the physiological state of the parental cells.

### Save time and get pure exosomes for your research

HansaBioMed's **purified lyophilized exosomes** are obtained from different biological sources including human biofluids and supernatants from a large number (over 100) of tumor cell lines (cell lysates are also available).

Lyophilized HBM-Exosome Standards	
Code	Source
<b>Lyophilized Exosomes from Human Biofluids</b>	
HBM-PEP	Human Plasma from pools of healthy donors
HBM-PES	Human Serum from pools of healthy donors
HBM-PEU	Human Urine from pools of healthy donors
HBM-PESL	Human Saliva from pools of healthy donors
<b>Lyophilized Exosomes from Cell Supernatants</b>	
HBM-COLO	Human Colon Carcinoma cell lines
HBM-MM1	Human Melanoma cell lines
HBM-BLCL21	EBV transformed lymphoblastoid B cell line

More than 100 additional cell lines are available for exosome purification or cell lysates on request. Check the list in HBM catalog and website ([http://exotest.eu/online\\_orders/standards](http://exotest.eu/online_orders/standards)).

Lyophilized Exosome Standards are sold in vials containing 100 µg or 30 µg of total protein (100 µg: Particles/ml > 1x10<sup>10</sup>. 30 µg: Particles/ml > 1x10<sup>8</sup>)

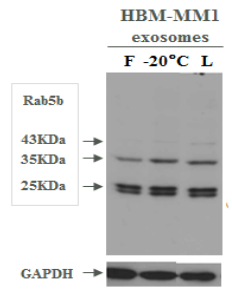
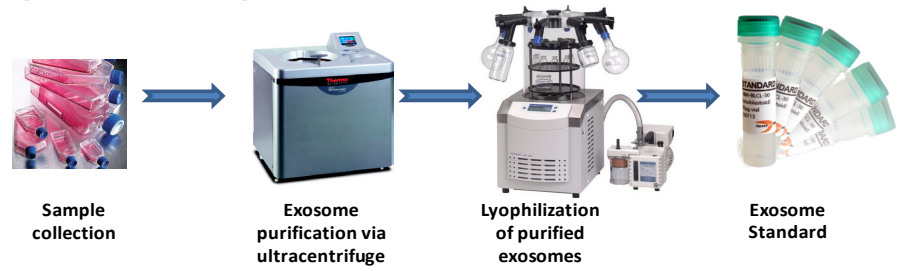
### Use HBM Exosome standard for...

- Assay calibration
- Control (spike-in) for exosome quantification
- Protein marker analysis using different techniques
- Extraction and analysis of exosomal nucleic acid
- Standardized positive controls for immunocapture performance evaluation

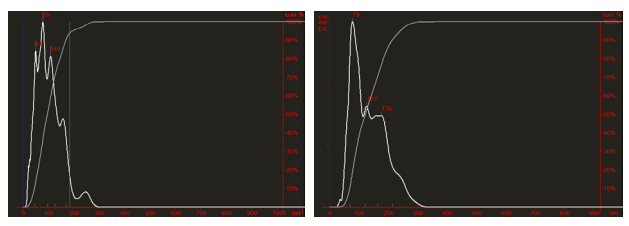
### Advantages

- High quality purified exosomes
- Easy to reconstitute
- Easy to ship and store (+4°C)
- Long term storage stability (36 months)
- Exosomes available from a large cell line bank (over 100 cell lines)
- Exosomes available from different body fluids (Plasma, Serum, Urine, Saliva)

## Lyophilization is the ideal method for preparing and preserving exosome stability for long-term storage at +4°C



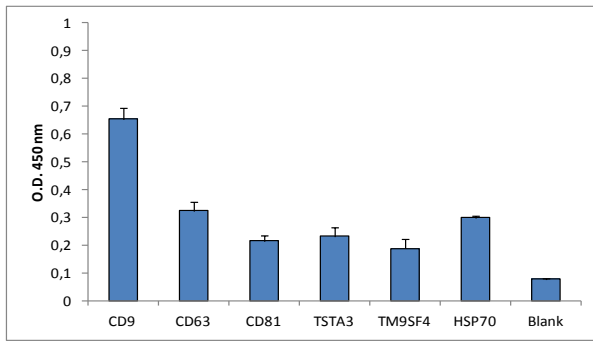
1. Western Blot comparison of exosomal markers on fresh (F), frozen (-20°C) and lyophilized exosomes (L)



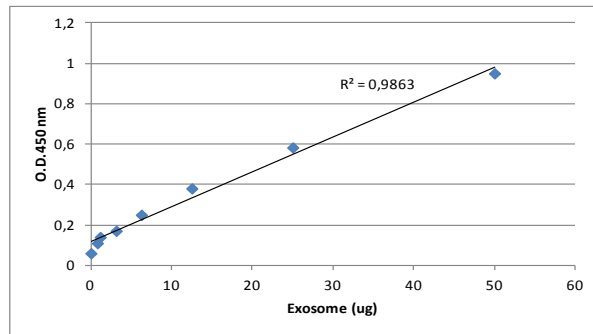
2. Comparative NanoSight analysis of freshly purified (right panel) and lyophilized plasma exosomes (left panel).

Lyophilized exosomes are easy to ship and store. Exosomes are isolated through a combination of ultracentrifugation and microfiltration procedures. Exosomes are subsequently quantified and validated for overall protein content and particle number by Nanoparticles Tracking Analysis (NTA, NanoSight). Lyophilization does not alter the stability of exosomal proteins and nucleic acids, in comparison to other storage methods, including storage of fresh exosomes at -20°C. Figures 1 and 2 compare the effect of lyophilization vs freezing at -20°C, showing no difference in protein expression at WB and exosome integrity at NanoSight respectively.

## HBM-Exosome Standards are suitable for many applications in exosome research

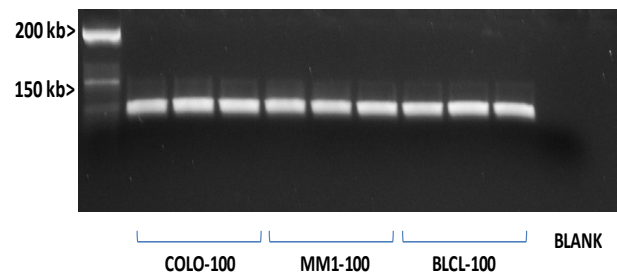


3. Expression of common exosomal markers on plasma purified exosomes (HBM-PEP-30)



4. Titration of HBM-Exosome Standard COLO-100 with anti-CD9 antibody in HBM-ImmunoPlate for Overall exosome capture from cell supernatants (HBM-POC)

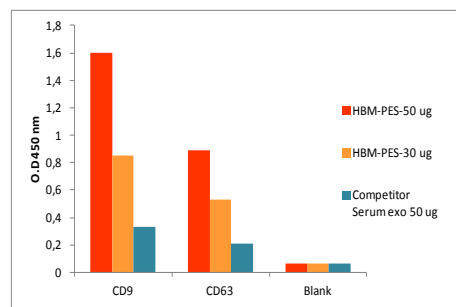
HBM Exosome Standards are suitable for many different applications and techniques such as ELISA assay, FACS, western blotting and NTA. Figures 3, 4 and 5 show some applications of our lyophilized exosomes. Figure 3 shows a profile of common protein markers in exosomes purified from human plasma. **HBM-PEP-30** standards are used for detection of each marker in ELISA assay, by using **HBM immunoplate** for Overall exosome capture from human plasma. In Figure 4 **HBM-COLO-100** are used to generate a standard curve suitable for exosome quantification by ELISA assay (**HBM-ExoTEST™**). In addition, HBM Exosome Standards are suitable for exosomal nucleic acid extraction and downstream analyses. Figure 5 shows amplification of  $\beta$ -Actin transcript from total RNA obtained from **HBM-COLO-100**, **HBM-MM1-100** and **HBM-BLCL21-100** standards. RNA extraction was performed using the **HBM-RNA extraction kit**.



5.  $\beta$ -Actin transcript amplification from total RNA extracted from HBM-Exosome Standard COLO-100, MM1-100, BLCL-100

## HBM Exosome Standards guarantee higher purity and performances at a lower price than similar products

Comparison of the HBM Exosome Standards from human serum (**HBM-PES**) vs human serum exosomes sold by a competitor: 50  $\mu$ g of each purified exosomes standard was analyzed on ELISA to detect exosomal markers (CD9 and CD63). Figure 6 shows higher signal for HBM exosomes (for both 50 and 30  $\mu$ g) than Competitor's exosomes (50  $\mu$ g). Exosome count by NTA (NanoSight) confirmed these results, revealing a significantly higher count of exosomes using HBM Exosome Standard (Figure 7).



6. CD9 and CD63 ELISA quantification of HBM Exosome Standard from Human Serum and similar Exosome purified by a Competitor

NTA particles/ml (p/ml)	
<b>HBM-PES (50 ug)</b>	<b>Competitor Serum Exo (50 ug)</b>
3,75x10 <sup>10</sup> p/ml	2,29x10 <sup>9</sup> p/ml

7. Exosome count by NTA (NanoSight) for the same amount (50  $\mu$ g) of HBM Exosome Standard from Human Serum (HBM-PES) and similar Exosomes purified by a Competitor

Features	HansaBioMed Exosome Standard	Competitor Exosome Standard
Amount for each preparation	100 ug	50 ug
Method of isolation	Ultracentrifuge	Precipitation Reagent
Nanoparticles/ml (average)	> 1x10 <sup>10</sup>	>1x10 <sup>6</sup>
Final form	Lyophilized	Frozen
Storage temperature	4°C (lyophilized)	-20°C
Expire time	36 months	24 months
Price (1 vial)	125 € x 100 ug	over 350 € x 50 ug

HBM Exosome Standards are purified using a protocol combining **ultracentrifugation** and **microfiltration steps** that guarantees the highest purity/integrity and the lowest contamination with other microvesicles. Competitor's Exosomes are isolated by chemical precipitation, therefore other microvesicles, protein-protein or protein-RNA complexes, and cell debris, regardless of their origin, contaminate the sample affecting its purity.

## Lyophilization and Purification Service

In addition to the products described in this leaflet HansaBioMed also provides services for exosome purification, quantification and analysis. We can facilitate your research with professional services performed by scientists experienced in the exosome field and using state of art equipment. A wide range of services is offered, from simple exosome purification and quantification at NanoSight and ExoTEST to biomarker discovery or other contract research activities.