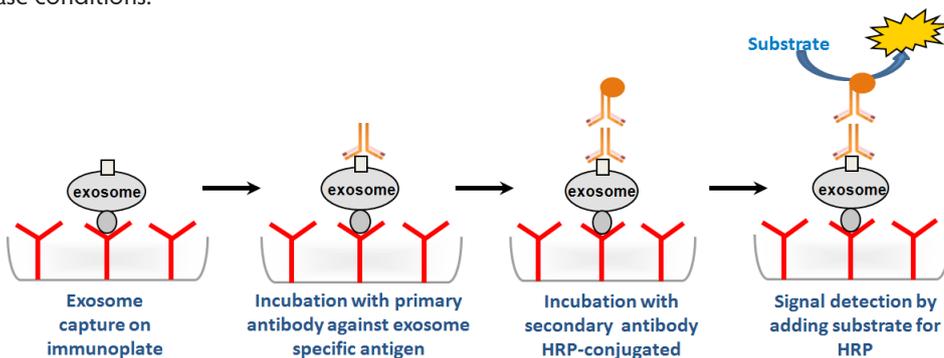




Double sandwich ELISA assay for exosome immunocapture and quantification from human biofluids or cell culture media

ExoTEST™: Ready to use kit for exosome capture and quantification

ExoTEST™ is a patented double sandwich ELISA assay for quantitative and qualitative analysis of exosomes. In particular ExoTEST™ is a successful platform for exosomes quantification and characterization from small amount of human biological fluids or cell media and it may be exploited to identify exosomes released by cancer cells in the plasma and urine of tumor patients in various disease conditions.



ExoTEST™ consists in ELISA plates pre-coated with proprietary pan-exosome antibodies enabling specific capture of exosomes from different biological samples, including cell culture supernatants and human biological fluids. Quantification and characterization of exosomal proteins is subsequently performed using appropriate detection antibodies against exosome associated antigens that can be for either generic or cell/tissue-specific exosomes. Lyophilized Exosome Standards, characterized for protein content and particle number (NTA) allow the quantification of unknown sample by a standard calibration curve.

HBM provides different types of ExoTEST™ kit for overall or specific exosome subpopulation capture and quantification

Cat. Code	Description
ExoTEST™ kits for Overall Exosome immunocapture and quantification	
HBM-RTK-POF/##	ExoTEST™ Ready to Use Kit for Overall exosome capture and quantification from human plasma and urine.
HBM-RTK-POS/##	ExoTEST™ Ready to Use Kit for Overall exosome capture and quantification from human serum.
HBM-RTK-POC/##	ExoTEST™ Ready to Use Kit for Overall exosome capture and quantification from cell culture media.
ExoTEST™ for Tumor-derived Exosome immunocapture and quantification from human plasma	
HBM-RTK-PTF/##	ExoTEST™ Ready to Use Kit for Tumor-derived exosome capture and quantification from human plasma.
Kits are also available in TEST format for testing, limited to 24 wells (3 ELISA strips). Code HBM-TRTK-###	

Applications

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

Advantages

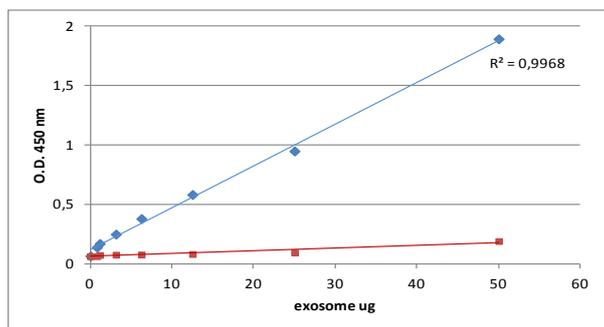
- Ready to use.
- No initial exosome purification required.
- User friendly and suitable for multiple marker analyses.
- Available in TEST format (limited to 3 ELISA strips, 24 wells).

HBM provides several ExoTEST™ kits for quantification of overall or specific exosome populations from human biofluids (plasma, urine, serum) and from cell culture supernatants. ExoTEST™ are also available with specific immunoplates for colorimetric (transparent), luminometric (white) or fluorimetric (black) readings. Customized ExoTEST™ kits can be provided for special research needs or for OEM productions.

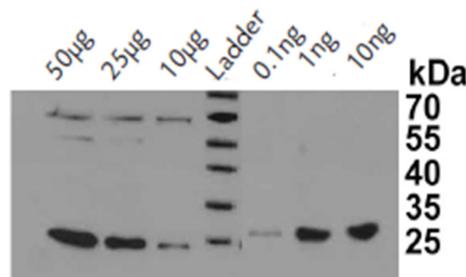
Tetta, Ciro, et al. "Extracellular vesicles as an emerging mechanism of cell-to-cell communication." *Endocrine* 44.1 (2013): 11-19.
 Zarovni, Natasa, et al. "Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches." *Methods* (2015).
 Vishnubhatla, Indira, et al. "The Development of Stem Cell-Derived Exosomes As a Cell-Free Regenerative Medicine." *J. Circ. Biomark* 143 (2014):
 .Logozzi, Mariantonia, et al. "High levels of exosomes expressing CD63 and caveolin-1 in plasma of melanoma patients." *PloS one* 4.4 (2009): e5219.

ExoTEST™ shows high sensitivity in detecting low exosome amount, high affinity and low background for exosome binding and quantification.

The sensitivity of the ExoTEST™ was compared to Western blot. The sensitivity of the ExoTEST™ is higher than Western blotting as reported in Figures 1 and 2 showing that 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. In addition, immunoplates used for exosome capture shows a low background which makes it a reliable, efficient and accurate test (Fig 1).

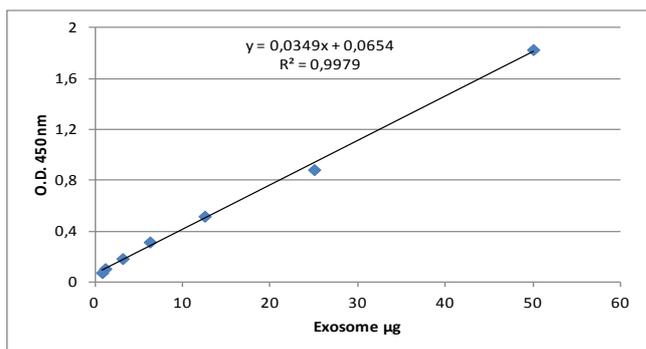


1. CD9 titration (blue line) of plasma healthy donor exosome standards (HBM-PEP100) and comparison with observed background (red line, only secondary antibody).



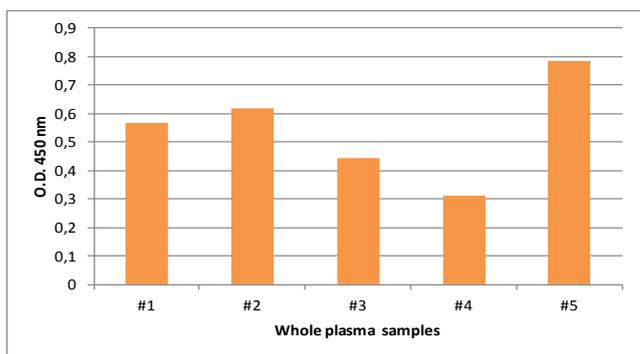
2. CD9 exosome marker detection by Western Blotting on lyophilized exosomes from human plasma (HBM-PEP100) and recombinant CD9 protein.

ExoTEST™ is a sensitive method for exosome quantification in human biofluids.



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

ExoTEST™ enables robust and precise quantification of exosomes from human biofluids, exhibiting consistency among individual samples and different experiments. Standard exosome preparations are provided in the kit to design standard curves and for assay calibration. We report an example of exosome quantification performed in 5 unknown plasma samples from healthy donors using the ExoTEST™ Ready to use kit for Overall Exosome quantification from human plasma (HBM-RTK-POF/TP). Following Lyophilized Standards and unknown samples binding onto the ELISA plate, test is run in according to the kit protocol and exosome detection is performed with anti-CD9 antibody (HBM).



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

Plasma sample	O.D. 450 nm	Exosome 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

5. Exosome quantification is performed calculating the quantity of exosomes (expressed in µg) into the 5 unknown samples through the equation of the standard curve (Fig 3). The particles number contained in 100 µl of plasma is calculated from quantity of exosomes (expressed in µg) in according to the particles concentration (number of particles/ml) indicated in the label of the Lyophilized Exosome Standards (HBM-PEP100, NTA: 3x10¹¹ particles/ml)

Custom made ExoTEST™ Ready to Use Kit

HansaBioMed offers the potential to design and create your own kit for dedicated applications choosing among a wide variety of reagents available in our catalog and beyond:

- 1- Select the plate that provides the specific capture or enrichment of exosomes of interest
- 2- Select the most appropriate exosome standard
- 3- Select the primary antibody for exosome biomarker detection