Lyophilized Exosome Standards

1- About Exosomes:

Exosomes are EVs actively secreted by exocytosis by most living cells, typically with diameter reported between 40 and 120 nm. Exosome release occurs either constitutively or upon induction, under both normal and pathological conditions. Both quantity and molecular composition of released exosomes depend on the physiological state of the parental cells.

2- Lyophilized Exosomes Standards:

Lyophilized Exosomes are purified by a combination of tangential flow filtration (TFF), size exclusion chromatography (SEC). Isolated vesicles are quantified and validated for total protein content, common marker expression (CD9, CD81,CD63), particle size distribution and concentration by NTA (Nanoparticles Tracking Analysis) with Zetaview analyzer (Particle Metrix). Lyophilization does not alter the stability of exosome proteins and nucleic acids, in comparison to other storage methods, including storage of fresh exosomes at -20°C. Lyophilized exosomes are easy to ship and stable for long term storage (up to 36 months).

3- Types of Exosome Standards available:

- Lyophilized Exosome Standards from human Biofluids (plasma, serum, urine, saliva) of healthy donors.
- Lyophilized Exosomes from cell culture media (COLO1, MM1, BLCL21, HCT116, SK-N-SH, U87, PC3, BPH-1, DAUDI, A549, K562, mouse cell B16F10).
- Lyophilized Exosomes from Human Mesenchymal Stem Cells (MSC) from adipose tissue. Pool of 10 different donors.
- Lyophilized Exosome Standards size avalable: 100 µg and 30 µg, sold in packages of 2, 5 vials.

4- Procedure for Exosome Standards reconstitution:

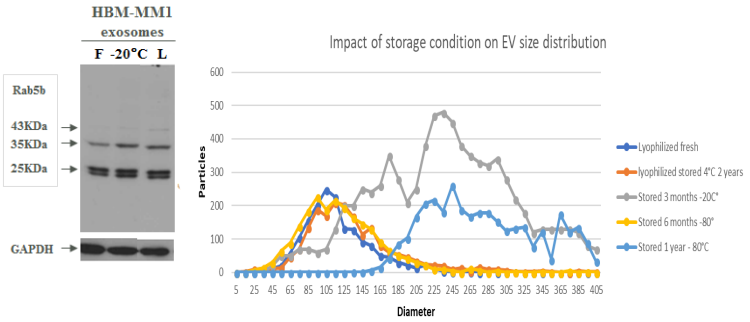
- Reconstitute Lyophilized Exosome Standard by adding deionized water, 100 µl for Lyophilized Standard 100 µg and 30 µl for Lyophlized Standard 30 µg, to get a final concentration of 1 µg/µL. Different volumes of deionized water for exosomes reconstitution can be choosen by the users in according with the desired final concentration. Resuspend exosomes pipetting the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted standard for 60 seconds.
- Briefly centrifuge the tubes containing the standard to ensure that the solution is collected at the bottom of the tube. Pipette the solution up and down 10 times, avoiding the introduction of bubbles. After this step, the standard is ready to use.

5- Storage:

- Lyophilized Exosomes can be stored for 36 months at 4°C.
- Reconstituted Exosome Standards are not suitable for long term conservation at room temperature; use them within 2 hours after reconstitution. The remaining reconstituted solution should be aliquoted into polypropylene vials (preferably low binding) and stored at -20°C for up to one month or at -80°C for up to six months. Strictly avoid repeated freeze-and-thaw cycles.

6- Performance:

Lyophilization does not affect EV particle size distribution or biomarker expression compared to other storage methods (Fig 1). Exosomes stored for over 3 months at -20° C or over 1 year at -80° C showed a different size distribution profile, probably due to EV aggregation.

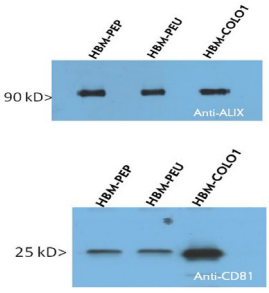


1. Western Blot comparison of exosomal markers on fresh (F), frozen (-20°C) and lyophilized exosomes (L). Particle size distribution of Exosomes stored lyophilized or frozen.

7- Application of Lyophilized Exosome Standards:

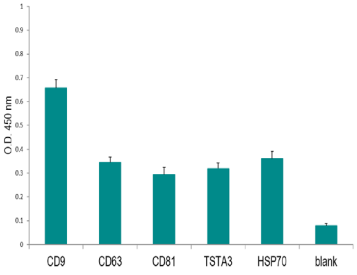
7a- Marker detection by Western Blotting.

Reconstituted Exosomes can be directly lysed in Laemmli buffer, then loaded on the Electrophoresis gel.
Recommended quantity: 10-20 µg per line.



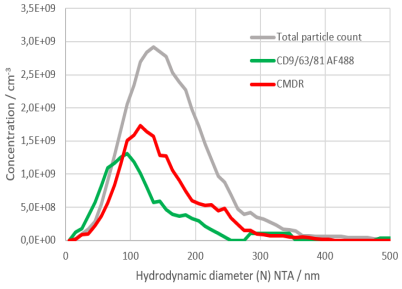
7b- Phenotyping by ELISA assay.

Reconstituted Exosomes can be loaded directly onto ELISA plate wells.
Recommended quantity: 10 - 20 µg per well.



7c- NTA in fluorescence and scattered mode.

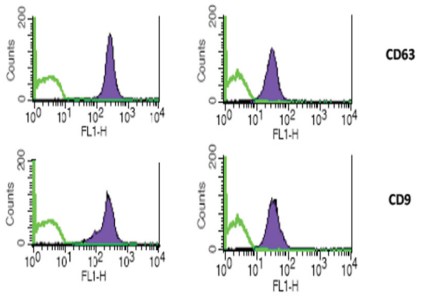
Reconstituted Exosomes can be used for phenotyping assays by fluorescence NTA.
Recommended quantity: 10 µg of Exosomes incubated with 1 µg* of antibody fluorophore conjugated or 2 µl* of membrane dye CMDR.



* Right ratio Exosome/dye have to be determined by the user.

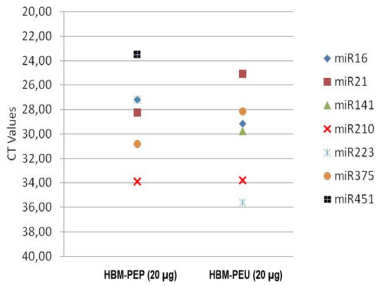
7d- Phenotyping assays by FACS.

Reconstituted Exosomes can be used for profiling biomarkers by FACS analysis.
Recommended quantity: 5 µg of reconstituted Exosomes Standards for each test.



7e- Profiling of Exosome associated nucleic acids.

Reconstituded Exosomes can be used for profiling nucleic acids (RNAs and DNA) biomarkers. The lysis buffer can be added directly to the Exosome preparation.
Recommended quantity: 20-50 µg per reaction.



8- References:

Giampieri, R., Piva, F., Occhipinti, G., Bittoni, A., Righetti, A., Pagliaretta, S., ... & Ricci, G. (2019). Clinical impact of different exosomes' protein expression in pancreatic ductal carcinoma patients treated with standard first line palliative chemotherapy. PloS one, 14(5), e0215990.

Campos-Silva, C., Suárez, H., Jara-Acevedo, R., Linares-Espinós, E., Martinez-Piñeiro, L., Yáñez-Mó, M., & Valés-Gómez, M. (2019). High sensitivity detection of extracellular vesicles immune-captured from urine by conventional flow cytometry. Scientific reports, 9(1), 2042.

Cao, H., Zhou, X., & Zeng, Y. (2019). Microfluidic exponential rolling circle amplification for sensitive microRNA detection directly from biological samples. Sensors and Actuators B: Chemical, 279, 447-457.

Dehghani, M., Gulvin, S. M., Flax, J., & Gaborski, T. R. (2019). Exosome labeling by lipophilic dye PKH26 results in significant increase in vesicle size. bioRxiv, 532028.

Woith, E., & Melzig, M. F. (2019). Extracellular Vesicles from Fresh and Dried Plants—Simultaneous Purification and Visualization Using Gel Electrophoresis. International journal of molecular sciences, 20(2), 357.

Ohara, M., Ohnishi, S., Hosono, H., Yamamoto, K., Yuyama, K., Nakamura, H., ... & Sakamoto, N. (2018). Extracellular Vesicles from Amnion-Derived Mesenchymal Stem Cells Ameliorate Hepatic Inflammation and Fibrosis in Rats. Stem cells international, 2018.

Piacenza, F., Biesemeier, A., Farina, M., Piva, F., Jin, X., Pavoni, E., ... & Basso, A. (2018). Measuring zinc in biological nanovesicles by multiple analytical approaches. Journal of Trace Elements in Medicine and Biology, 48, 58-66.

Kabe, Y., Suematsu, M., Sakamoto, S., Hirai, M., Koike, I., Hishiki, T., ... & Minegishi, N. (2018). Development of a Highly Sensitive Device for Counting the Number of Disease-Specific Exosomes in Human Sera. Clinical chemistry, clinchem-2018.

Shi, L., Rana, A., & Esfandiari, L. (2018). A low voltage nanopipette dielectrophoretic device for rapid entrapment of nanoparticles and exosomes extracted from plasma of healthy donors. Scientific reports, 8.

Dominkuš, P. P., Stenovec, M., Sitar, S., Lasič, E., Zorec, R., Plemenitaš, A., ... & Lenassi, M. (2018). PKH26 labeling of extracellular vesicles: characterization and cellular internalization of contaminating PKH26 nanoparticles. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1860(6), 1350-1361.

Valkonen, S., van der Pol, E., Böing, A., Yuana, Y., Yliperttula, M., Nieuwland, R., ... & Siljander, P. R. M. (2016). Biological reference materials for extracellular vesicle studies. European Journal of Pharmaceutical Sciences.

Oliveira-Rodríguez, M., López-Cobo, S., Reyburn, H. T., Costa-García, A., López-Martín, S., Mo, M. Y., ... & Blanco-López, M. C. (2016). Development of a rapid lateral flow immunoassay test for detection of exosomes previously enriched from cell culture medium and body fluids. Journal of Extracellular Vesicles, 5.

Arcangeletti, M. C., Simone, R. V., Rodighiero, I., De Conto, F., Medici, M. C., Maccari, C., ... & Calderaro, A. (2016). Human cytomegalovirus reactivation from latency: validation of a "switch" model in vitro. Virology Journal, 13(1), 179.

Zhang, Peng, Mei He, and Yong Zeng. "Ultrasensitive Microfluidic Analysis of Circulating Exosomes Using Nanostructured Graphene Oxide/Polydopamine Coating." Lab on a Chip (2016).

Sitar, Simona, et al. "Size characterization and quantification of exosomes by asymmetrical-flow field-flow fractionation." Analytical chemistry (2015).

Gardiner, Chris, et al. "Measurement of refractive index by nanoparticle tracking analysis reveals heterogeneity in extracellular vesicles." Journal of extracellular vesicles 3 (2014).

Ferrante, Sarah C., et al. "Adipocyte-derived exosomal miRNAs: a novel mechanism for obesity-related disease." Pediatric research 77.3 (2014): 447-454.

HansaBioMed Life Sciences Ltd
Mäealuse 2/1
12618, Tallinn (EE)
Email: info@hansabiomed.eu
Tel: +372 6561996
www.hansabiomed.eu

Investors