ExoStd™Lyophilized Exosome Standard (100 µg, Human Saliva)

Catalog # M1047-2, -6 (100 µg; Store at 4°C)

I. Introduction:

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 the amount and molecular composition of released exosomes depend on the state of a parent cell. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Exosomes have pleiotropic physiological and pathological functions and an emerging role in diverse pathological conditions such as cancer, infectious and neurodegenerative diseases.

ExoStd™ lyophilized exosome standards are standardized positive controls for immunocapture performance evaluation. Lyophilization is the ideal technique for preserving the long-term stability of exosomes at 4ºC. Lyophilized exosomes can be used as control standards for multiple applications including FACS, WB, ELISA and as calibration standards for quantitation of exosome-derived markers from biological samples. Lyophilized exosomes are easy to ship and store, and are stable for over 36 months at 4°C. Purified and lyophilized exosomes are obtained from a variety of biological sources: cell culture supernatant, human plasma, serum, urine and saliva. Exosomes are purified following a combination of ultracentrifugation and microfiltration steps. Exosomes are subsequently quantified and validated for overall protein content and particle number by NTA (Nanoparticles Tracking Analysis) with NanoSight LM10. Lyophilization does not affect the stability of purified exosomes and the expression levels of their exosome markers (proteins and nucleic acids). ExoStd™ lyophilized exosome standards are highly pure, easy to reconstitute and easy to ship and store at 4ºC.

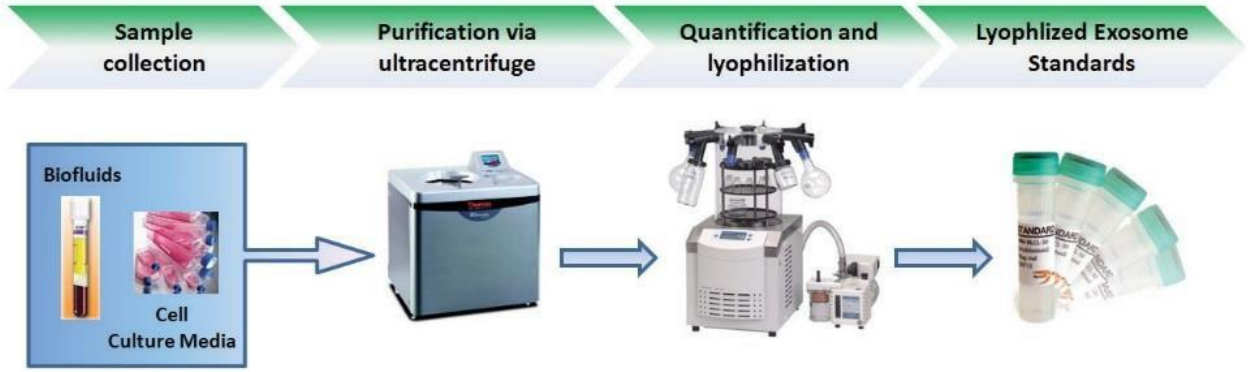

Figure 1. ExoStd™ Purified and Lyophilized Exosomes. Purified and lyophilized exosomes are obtained from a variety of biological sources: cell culture supernatant, human plasma, serum, urine and saliva. Exosomes are purified following a combination of ultracentrifugation and microfiltration steps.

II. Application:

- Assay calibration.
- Control (spike-in) for exosome quantification.
- Protein markeranalysis using different techniques.
- Extraction and analysis of exosome nucleic acid, Flow cytometry, Electron microscopy.

III. Sample Type:

- Lyophilized and purified Exosomes available from Human Saliva.

IV. Package Contents (Lyophilized and purified exosomes standards from human saliva of healthy donors):

M1047-2	**2 vials (2 x 100 µg)**
M1047-6	**6 vials (6 x 100 µg)**

V. User Supplied Reagents and Equipment:

- Deionized water

VI. Shipment and Storage:

- All the Lyophilized Exosome Standards are shipped at 4°C
- Lyophilized Exosome Standards can be stored at 4°C for up to 36 months.
- Reconstituted exosome standards are not suitable for long term conservation at room temperature, use them within 2 hours after reconstitution. The remaining reconstituted standard stock 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.

VII. Reagent Preparation and Storage Conditions:

- All the Lyophilized Exosome Standards are shipped at 4°C
- Reconstitute lyophilized exosome standard by adding deionized water.
- Reconstituted exosome standards are not suitable for long term conservation at room temperature, use them within 2 hours after reconstitution. The remaining reconstituted standard stock 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.

VIII. ExoStd™ Assay Protocol:

1. Reconstitute lyophilized exosome standard by adding deionized water. 100 µl for lyophilized standard 100 µg, to get a final concentration of 1 µg/µl. Different volumes of deionized water for exosomes reconstitution can be chosen by the users in according with the desired final concentration. Suspend exosomes pipetting the solution up and down 10-15 times, avoiding bubbles. Vortex the reconstituted standard for 60 secs.
2. 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.
3. **Reproducibility:** Lyophilization is the ideal method for preserving exosome stability. Expression of exosomal markers was assayed with different techniques (WB, FACS, ELISA) and nanovesicle count was measured with NTA. Lyophilization did not substantially affect exosome count or biomarker expression compared to other storage methods (Figures 2, 3, 4, 5). Comparing different storage methods of exosome standards (fresh vs. frozen vs lyophilized) with an anti-CD81 ELISA assay **(ExoQuant™)**, the loss of signal compared to fresh material is minimal when using lyophilized exosomes (CV - 15%) (Figure 5). Biovision Exosome Standards are the ideal tool for your exosome studies. Examples as shown in Figures 6, 7 shows a profile of common exosomal markers in human plasma exosomes (M1040-30), Figure 6) and Beta-actin transcript amplified from RNA obtained from COLO-100, MM1-100 and BLCL21-100 standards (Figure 7). Biovision Exosome Standards guarantee higher purity and better performances over competitors (Figures 8, 9).

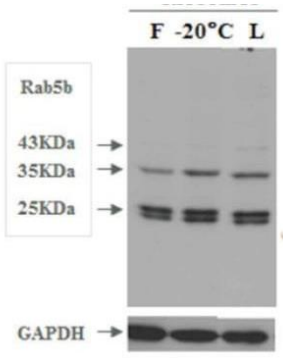

Figure 2. Western Blot comparison of exosomal markers on fresh (F), frozen (-20C) and lyophilized exosomes (L).

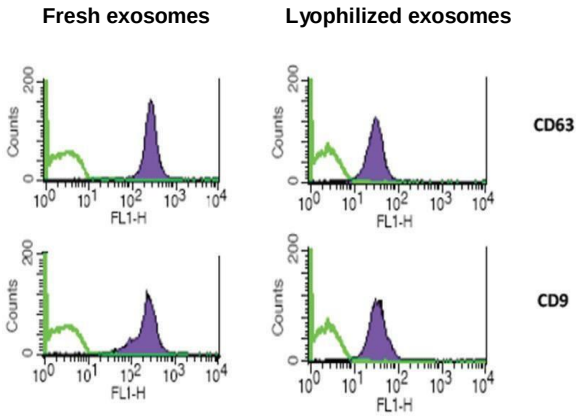

Figure 3. Comparison of exosomal markers on fresh and lyophilized exosomes (L).

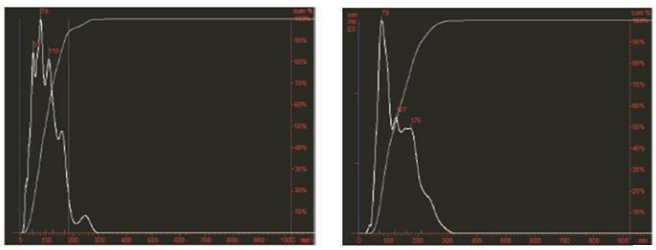

Figure 4. Comparative Nanosight analysis of freshly purified (lower panel) and lyophilized plasma exosomes (upper panel).

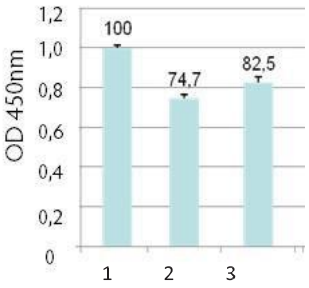

Figure 5. ExoQuant™ comparative detection of CD81 on Biovision- MM1 derived exosomes. 1- Fresh exosomes; 2- Frozen exosomes (-20°C); 3- Lyophilized exosomes.

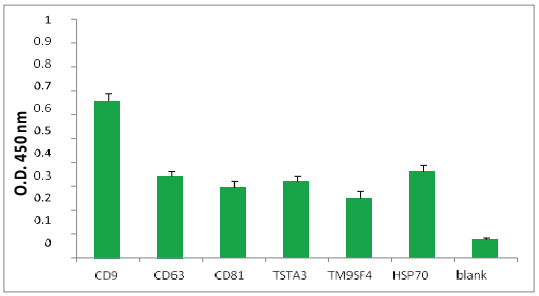

Figure 6. Profile of common exosomal markers in plasma exosome standard. Biovision (M1040-30) is used to quantify each tested marker.

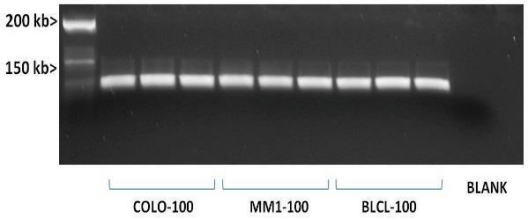

Figure 7. β-Actin transcript amplification from total-RNA extracted from Biovision Exosome Standard COLO-100, MM1-100, BLCL-100 marker.

Biovision Exosome Standard from human serum (M-1043) were compared to human serum exosome standard from a Competitor. Exosomal markers CD9 and CD63 from 50 µg of both serum exosome standards were detected using an ELISA assay (Figure 8). Biovision Standard (50 µg) generated the highest signal, whereas the signal from Competitor Standard (50 µg) was lower than 30 µg of Biovision Standard. In a second test, 50 µg of Biovision and Competitor's Standards were serially diluted to design a standard curve for exosome quantification (Figure 9). The standard curve generated with Biovision Standard was linear (R^2 = 0.9876) within the concentration range and suitable for exosome quantification.

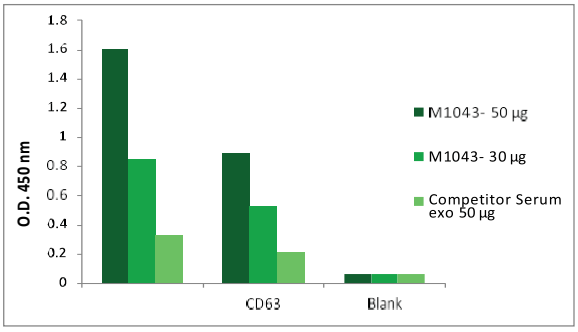

Figure 8. ELISA quantification of Biovision Exosome Standard vs Competitor's standard (human serum) for exosomal markers CD9 and CD63.

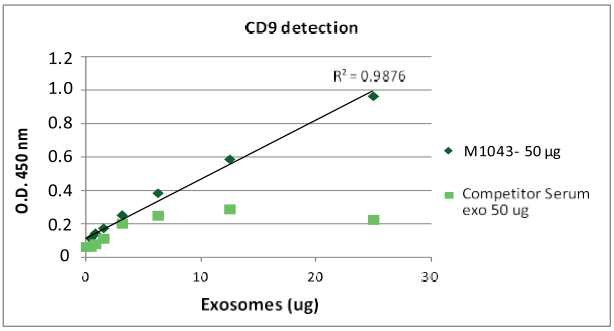

Figure 9. Standard curve for exosome quantification: Biovision Exosome Standard vs. Competitor's Standard.

Why to choose Biovision Exosome Standards:

Characteristics	Biovision Exosome Standards	Competitor's Exosome Standards
Amount per vial	100 µg	50 µg
Method of isolation	Ultracentrifuge	Precipitation Reagent
Nanoparticles/ml (average)	> 1x10^10	>1x10^6
Nanoparticles/ml in human serum exosomes (50 µg)	3.75x10^10 p/ml	2.29x10^9 p/ml
Final form	Lyophilized	Frozen
Storage temperature	4°C (lyophilized)	-20°C
Expire time	36 months	24 months
Price (1 vial)	*	***

Biovision Exosome Standards are purified using a combination of ultracentrifugation and microfiltration steps. This is a tedious and time-consuming methodology but it guarantees high purity of exosomes, with low contamination from other microvesicles, previously eliminated through microfiltration and differential centrifugation cycles. Conversely, chemical reagents currently used for vesicle isolation precipitate both exosomes and larger vesicles, protein-protein and protein-RNA complexes, regardless of their origin. This impairs the purity of the sample. Lyophilization also allows easy and longer term storage of purified exosomes.