ExoQuick-LP™ Exosome Isolation Kit

Cat. # EXOLPxA-1

User Manual

Store kit at +4 °C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

Contents

List of Components in ExoQuick-LP (Cat #EXOLP5A-1)

Item	Volume
ExoQuick exosome precipitation solution (1ml)	1 ml
Pre-Clearing Reagent A	25 µl
Pre-Clearing Reagent B	25 µl
Magnetic Bead Solution	500 µl
Wash Buffer	50 ml

Magnetic stand for pulling beads is not included in the kit, but can be purchased separately from SBI (Cat #EXOFLOW700A-1)

The ExoQuick-LP kit components are shipped on blue ice and should be stored at +4°C upon receipt. Pre-Clearing Reagent A and B can be stored at +4°C upon receipt for 1 month. For long term use, please store Pre-Clearing Reagent A and B at -20°C. Properly stored kits are stable for 1 year from the date received.

The kit provides enough ExoQuick (1 mL) for processing up to 500 µl of serum or plasma samples** good for 5 reactions, with each reaction defined as a single 100 µl sample.

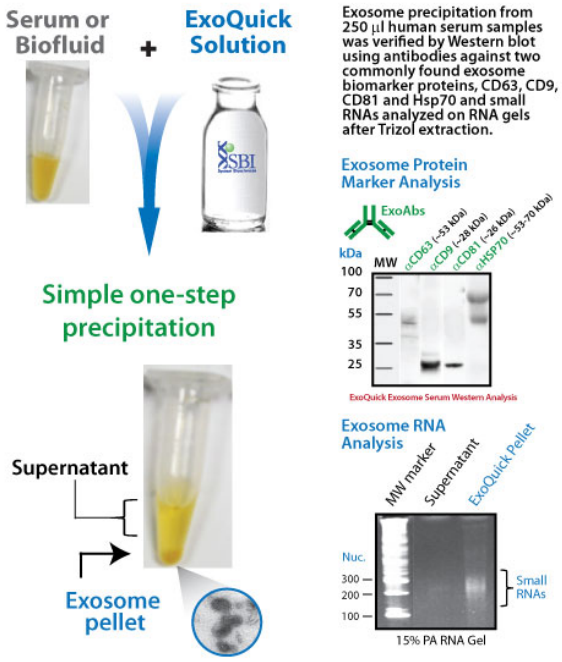
**If starting material is plasma, it must be pre-treated with SBI's Thromboplastin D reagent (please see protocol here: http://www.systembio.com/downloads/PAC_EXOQ5TD-1.pdf) prior to starting the ExoQuick-LP protocol.

ExoQuick Exosome Precipitation

I. Overview

Exosomes are 40 –150 nm membrane vesicles secreted by most cell types in vivo and in vitro. Exosomes are found in blood, urine, amniotic fluid, malignant ascite fluids and contain distinct subsets of microRNAs depending upon the tumor from which they are secreted. SBI's ExoQuick exosome precipitation reagent makes microRNA and protein biomarker discoveries simple, reliable and quantitative. Enrich for circulating exosomal microRNAs with ExoQuick™ and accurately profile them using SBI's SeraMir™ qPCR arrays.

* No time-consuming ultracentrifugation
* Less expensive than costly antibodies and beads
* More effective than any other method
* Use as little as 100 µl of serum or bio-fluid



Human blood plasma contains a heterogeneous mixture of microvesicles, exosomes, apoptotic bodies, and lipoprotein particles. These particles have been previously shown to carry nucleic acid cargo in the form of microRNAs (Vickers *et al*. 2012). Therefore, circulating microRNAs are a mixture of microRNAs present in the aforementioned subcellular particles. These microRNAs are known to be quite stable in blood plasma and the levels of specific circulating microRNAs can differ with diseases, leading to the possibility of using extracellular microRNAs as disease biomarkers.

In order to study only the exosome-associated microRNAs and proteins, it is important to remove as many subcellular particles as possible from blood plasma or serum before the treatment with ExoQuick, particularly lipoprotein particles. Lipoproteins are the major lipid-based particles in the plasma and serum. It has been recently demonstrated that both high-density lipoproteins (HDLs) and low-density-density lipoproteins (LDLs) contain microRNAs (Vickers *et al.* 2011). In order to deplete as much of these lipoproteins, which may cause false positive "hits" in analysis of exosome-associated microRNAs, we have developed the ExoQuick-LP kit containing ExoQuick and proprietory pre-clearing reagents to efficiently deplete lipoprotein particles from plasma or serum before ExoQuick precipitation.

II. ExoQuick-LP protocol

1. Use two 1.5 ml microcentrifuge tubes for one serum sample (100 µl)

   **If starting material is plasma, it must be pre-treated with SBI's Thromboplastin D reagent (please see protocol here: http://www.systembio.com/downloads/PAC_EXOQ5TD-1.pdf) prior to starting the ExoQuick-LP protocol.

2. Transfer 50 µl of magnetic bead suspension into each of the 1.5ml microcentrifuge tubes and add 300 µl of wash buffer to each tube and vortex briefly.
3. Place the tubes on a 1.5ml microcentrifuge tube magnetic separator and discard the supernatant after beads are collected. Repeat wash step with 300 µl of wash buffer.
4. Centrifuge tubes briefly, resuspend beads in 100 µl of wash buffer in each 1.5ml microcentrifuge tube. Add 5 µl of Pre-Clearing Reagent A to one tube and 5 µl Pre-Clearing Reagent B to another tube, respectively.
5. Incubate the two tubes with rotation for 30-60 minutes at room temperature.
6. Centrifuge the tubes briefly, place on a magnetic separator and pipette off the supernatant.
7. Repeat the wash step.
8. Remove tubes from the magnetic separator, add 200 µl of wash buffer to each tube and combine beads into one tube.
9. Centrifuge 0.5 ml of human serum sample at 3000 x g for 15 minutes to remove cells and cell debris and collect the supernatant.
10. Pass the above-mentioned supernatant through a 0.22 µm filter and collect the serum
11. Add 100 µl of filtered serum into the bead mixture.
12. Incubate the tube with rotation for 3 hours at 4^0C. After incubation, centrifuge the tube briefly and place on a magnetic separator.
13. Transfer the supernatant into a new tube, which contains lipoprotein-depleted serum.
14. Add ExoQuick (using 1/5 of the volume of the supernatant) into the tube containing supernatant and mix well by inverting the tube or shaking the tube up and down.
15. Incubate at 4^0C overnight.
16. Centrifuge the tube at 14000 rpm for 10 minutes next day.
17. Aspirate the supernatant, and the remaining pellet contains lipoprotein-depleted exosomes for downstream applications.

    Please refer to the full ExoQuick user manual (http://www.systembio.com/downloads/Manual_ExoQuick_WEB.pdf) for protocol suggestions on isolating protein and RNA from the pellet.

III. Sample Data for ExoQuick-LP

ApoA1 is the major protein component of high-density lipoprotein or HDL (comprising approximately 70% of the protein moiety of HDL). ApoE is a protein found mainly in low-density lipoprotein or LDL. ApoE is also found with a smaller amount in HDL.

Treatment of serum samples with Pre-Clearing Reagents A and B significantly reduces the amount of both ApoA1 and ApoE in ExoQuick serum pellet as measured by ELISA (Fig. 1).

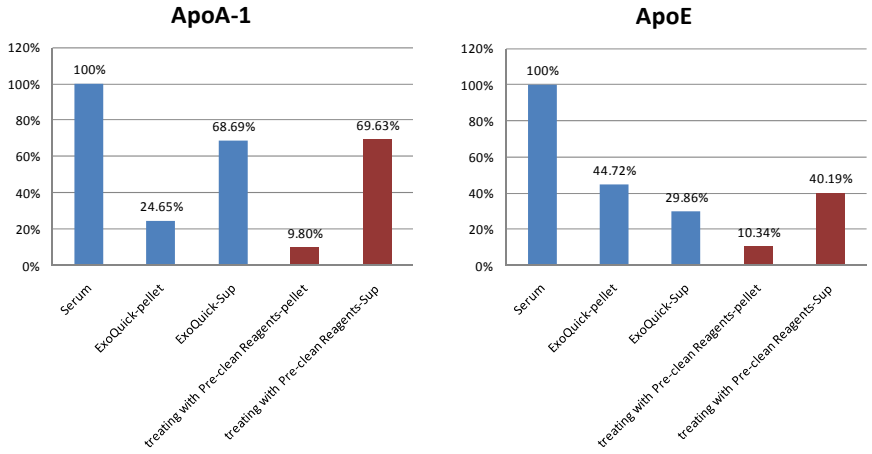


Figure 1. Validation data showing treatment of serum samples with SBI's ExoQuick-LP reagents leads to significant reduction of ApoA1 and ApoE lipoproteins

We used Exosome ELISA kits (SBI Cat. # EXOEL-CD81A-1, EXOEL-CD63A-1, and EXOEL-CD9A-1) to measure the approximate number of exosomal particles present in ExoQuick serum pellets with or without treatment with ExoQuick-LP Pre-Clearing Reagents (Fig. 2). While there were some differences in observed particle numbers in untreated vs treated samples, overall yields were quite similar, suggesting minimal loss of material using ExoQuick-LP.

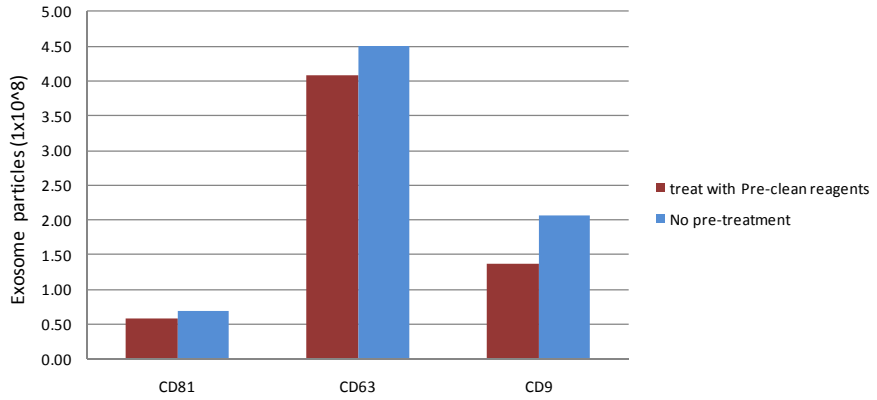


Figure 2. Validation data showing overall exosome particle numbers comparing ExoQuick pellets that were treated with ExoQuick-LP vs untreated control

To visually test for exosome integrity, we performed dynamic light-scattering analysis of untreated and ExoQuick-LP treated exosomes using the NanoSight LM10 instrumentation (see previous section for details). In brief, we passed 100 µl of serum through a 0.22 µm filter before treating with ExoQuick-LP as well as an untreated control. We also collected samples that were not filtered through a 0.22 µm filter for comparison. The ExoQuick serum pellets were resuspended in 100 µl PBS buffer and then diluted 1:2,000 before sending to Particle Characterization Lab for visualization and analysis. The results are shown as a plot of frequency vs particle size (Fig. 3). The data indicates that there are minimal differences in particle sizes using ExoQuick-LP vs untreated controls, suggesting that exosomes are structurally intact.

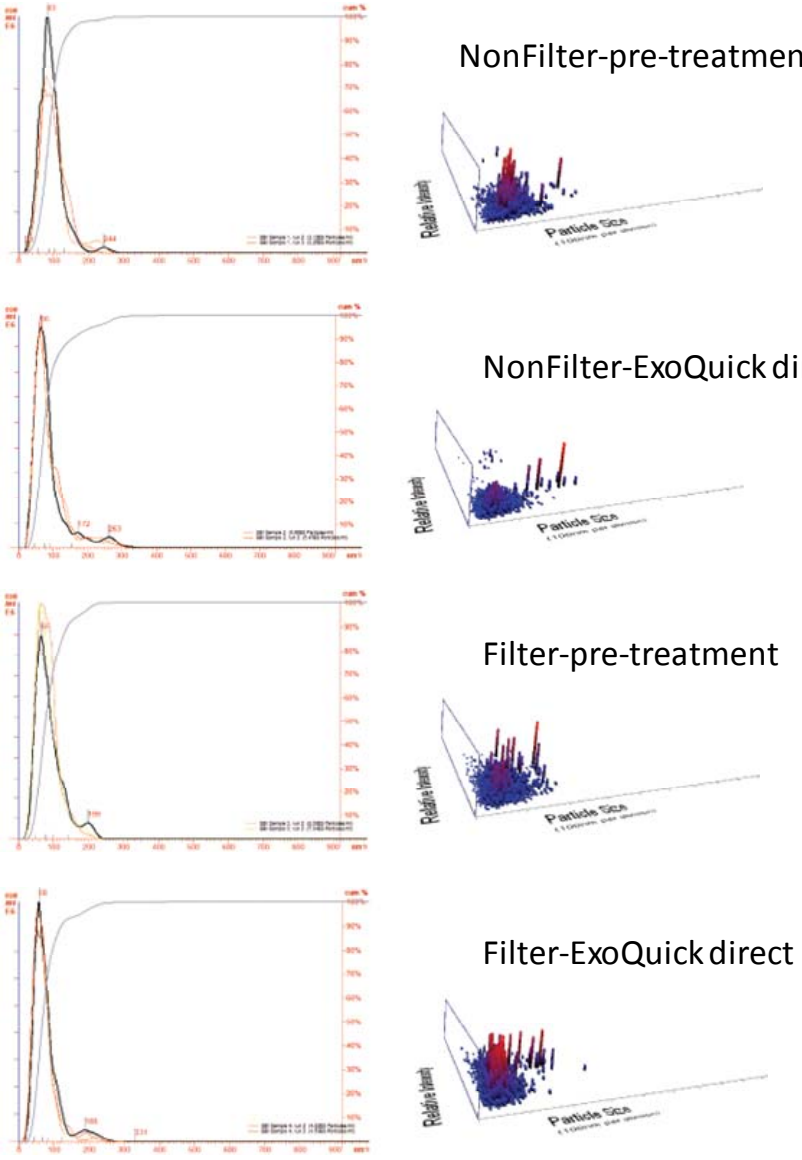


Figure 3. NanoSight analysis plots of frequency vs particle size in unfiltered and filtered samples that were untreated or ExoQuick-LP treated

IV. References

Vickers KC and Remaley AT. Lipid-based carriers of microRNAs and intercellular communication. Curr Opin Lipidol. 2012 Apr;23(2):91-7.

Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol. 2011 Apr;13(4):423-33.

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http://www.systembio.com

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