



ExoQuick-TC PLUS™

Exosome Purification Kit for Tissue Culture Media

Cat# EQPL10TC-1

User Manual

Store kit at +4°C

Version 2
2/22/2017

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

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Product Description

ExoQuick-TC PLUS™ is the latest solution for addressing protein carryover often found in exosomal preps from tissue culture media. It represents an evolution of traditional precipitation-based exosomal preps to address needs for downstream applications where exosomal purity is a concern (e.g. mass spectrometry, exosome labeling, *in vivo/ex vivo* exosome delivery).

The kit comes with ExoQuick-TC as well as our convenient, pre-packed Microsphere beads good for 10 reactions*. With less than 10 min of total hands-on time from precipitated exosomes to purified exosomes, ExoQuick-TC PLUS provides a simple, yet effective solution for obtaining high-purity, intact exosomes for most downstream applications.

***1 reaction is defined as exosomes resuspended in 250uL of Resuspension Buffer from 5mL of tissue culture media precipitated using ExoQuick-TC.**

List of Components

Item	Volume	Storage Temperature
ExoQuick-TC Reagent	10 mL	4°C
ExoQuick PLUS Microsphere beads	10 units	4°C
Resuspension Buffer	12 mL	4°C

Storage

The ExoQuick-TC PLUS kits are shipped at blue ice and should be **stored** at +4°C. Properly stored kits are stable for 6 months from the date received.

General Information

The reaction size is based on using 5 mL of tissue culture media sample.

Protocol for ExoQuick-TC PLUS

1. Collect biofluid and centrifuge at $3000 \times g$ for 15 minutes to remove cells and cell debris.
2. Transfer supernatant to a sterile tube and add the appropriate volume of ExoQuick-TC Exosome Precipitation Solution to the Biofluid. Some examples are shown in the table below. Mix well by inverting or flicking the tube.

Incubation Time	Biofluid	Sample Volume	ExoQuick TC volume
12 h- O/N	Conditioned Culture Medium	5mL	1mL

3. Incubate overnight (at least 12 hours) at $4\text{ }^{\circ}\text{C}$. The tubes do not need to be rotated during the incubation period.
4. Centrifuge ExoQuick-TC/biofluid mixture at $1500 \times g$ for 30 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the tube.
5. Carefully aspirate off supernatant. Spin down residual ExoQuick-TC solution by centrifugation at $1500 \times g$ for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated exosomes in pellet.
6. Resuspend exosome pellet in 250uL of Resuspension buffer.
7. Add 400uL of Resuspension buffer to 1 unit of Microsphere beads. Shake/vortex the solution for 3 minutes.
Note: 1 unit of beads is sufficient for exosomes isolated from 5 mL conditioned medium only. Scale up according to the volume of media used for exosome isolation.
8. Spin down the beads at $3000 \times g$ for 3 minutes.
9. Remove supernatant and repeat the wash with Resuspension buffer for a total of two washes (step 7 and 8).
10. Remove all the liquid, and add 250uL of your sample (from step 6) directly to the washed beads.
11. Mix at room temperature on an inverting shaker for 15 minutes.
12. Spin your sample for 5 minutes at $8000 \times g$.
13. The supernatant contains purified exosomes.

Next Steps and Related Products

Application	Related Products	Website links
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck™ Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
Quantification of Exosomes		
Quantification of exosomes	FluoroCet assay	https://www.systembio.com/quantitate-exosomes/fluorocet
Quantification of exosomes	ExoELISA-ULTRA assay	https://www.systembio.com/quantitate-exosomes/exoelisa-ultra
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir™ kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

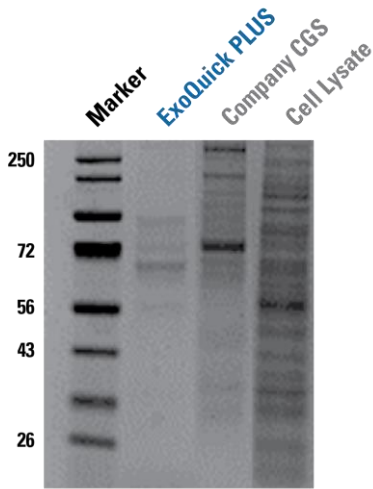


Figure 1. Total protein isolated from bovine-exosome depleted media (Exo-FBS) using ExoQuick-TC PLUS show decreased protein carry-over compared to competitor's product. For each lane, 5 μ g of total protein was loaded. After running the gel, total protein was detected using colloidal blue staining. *Data courtesy of Richard J. Jones, PhD., MD Anderson Cancer Center.*

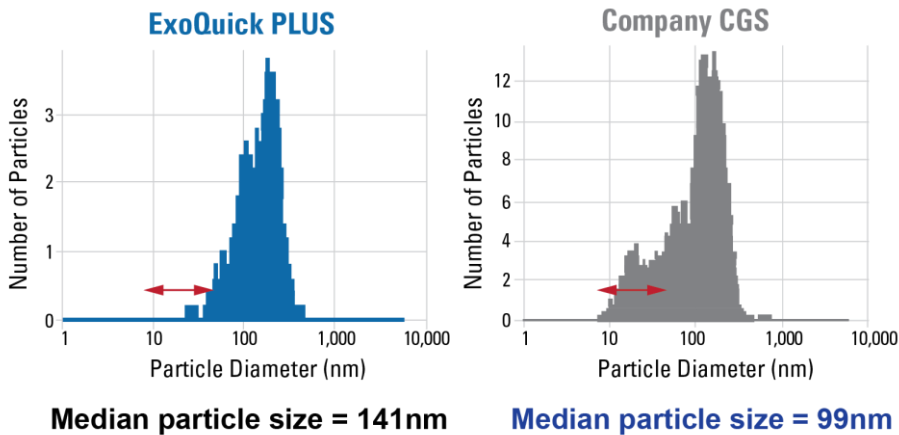


Figure 2. Exosome isolation with ExoQuick-TC PLUS results in higher purity preps. ZetaView particle size analysis of exosomes isolated from cultured RPMI 8226 cells using either ExoQuick-TC PLUS (left panel) or a competitor's kit (right panel). The red double arrow in the left panel indicates a broad shoulder of particles too small to be exosomes (< 50 nm in diameter) which are likely to be undesirable protein aggregates. These contaminants are not present in the exosomes isolated using ExoQuick-TC PLUS (red double arrow indicates the same particle diameter range as in the right panel). *Data courtesy of Richard J. Jones, PhD., MD Anderson Cancer Center.*

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: <http://www.systembio.com>

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Licensing and Warranty Statement

Limited Use License

Use of the ExoQuick-TC PLUS Exosome Purification Kit (*i.e.*, the “Product”) is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

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- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

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