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ExoQuick-TC™

Exosome Precipitation Solution

Cat# EXOTC10A-1

Cat# EXOTC50A-1

User Manual

Store kit at room temperature (+25°C) or 4°C on receipt

Version 8
11/14/2016

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™ is a proprietary polymer that gently precipitates exosomes and microvesicles between 30 and 200 nm in size from tissue culture media, urine, or spinal fluid. First, pre-clear your samples of cells and cellular debris, and then simply add the appropriate amount of ExoQuick-TC to your cleared biofluid, refrigerate, and centrifuge (see the product manual for protocol details). Your exosomes will be in the pellet, ready for resuspension in an appropriate solution.

List of Components

Item	Catalog #	Volume	Reactions
ExoQuick-TC exosome precipitation solution	EXOTC10A-1	10 ml	Up to 10 reactions
ExoQuick-TC exosome precipitation solution	EXOTC50A-1	50 ml	Up to 50 reactions

Storage

The ExoQuick-TC™ kits are shipped at room temperature, blue ice or dry ice and should be **stored** at +4°C or room temperature upon receipt. Properly stored kits are stable for 1 year from the date received.

General Information

The reaction size is based on using 5 ml of tissue culture media or urine for exosome isolation. For best recovery for both RNA and Protein analysis, we recommend starting with 10 ml sample. Examples of precipitating exosomes from various biofluids can be seen in the Table below.

Bio-fluid	Sample volume	ExoQuick-TC volume
Tissue Culture media	5 ml/10 ml	1 ml/2 ml
Urine	5 ml/10 ml	1 ml/2 ml
Spinal fluid	5 ml/10 ml	1 ml/2 ml

To isolate exosomes **from serum**, we recommend using the **ExoQuick** reagent (cat# EXOQ5A-1 or EXOQ20A-1) which is a distinct formulation from ExoQuick-TC reagent detailed in this manual.

To isolate exosomes from **plasma**, we recommend using the **ExoQuick Plasma Prep and Exosome Precipitation Kit** (Cat# EXOQ5TMA-1). Plasma contains fibrin which will precipitate along with ExoQuick causing an insoluble pellet to form. The ExoQuick Plasma Prep and Exosome Precipitation kit contains reagents to help dissolve the fibrin, thus increasing the yield of exosomes precipitated.

Protocol: ExoQuick-TC™

Note: This protocol is a starting point for most cells in culture. Different cell types secrete different amount of exosomes. You may need to scale this protocol accordingly.

1. Collect biofluid and centrifuge at $3000 \times g$ for 15 minutes to remove cells and cell debris.
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick-TC to the bio-fluid. Mix well by inverting or flicking the tube.
3. Refrigerate overnight (at least 12 hours) at $+4^{\circ}\text{C}$. The tubes should not be rotated or mixed during the incubation period and should remain upright.
4. Centrifuge ExoQuick-TC/biofluid mixture at $1500 \times g$ for 30 minutes. Centrifugation may be performed at either room temperature or $+4^{\circ}\text{C}$ with similar results. After centrifugation, the exosomes may appear as a beige or white pellet at the bottom of the vessel.

Exosome pellets obtained from 10 ml of cerebral spinal fluid using ExoQuick.



5. Aspirate 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 100-500 μl using sterile 1X PBS, or **specific buffer according to your downstream application**. We recommend using the precipitated exosomes immediately rather than freezing them for future use.

Next Steps and Related Products

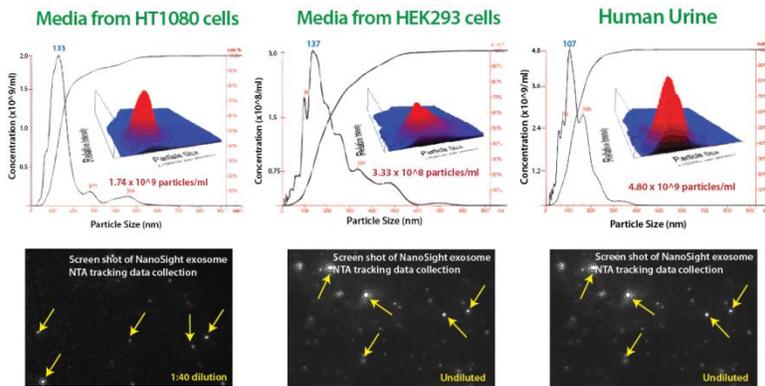
Application	Related Products	Website links
Precipitation of Exosomes from other biological fluids		
Exosome Isolation from Serum	ExoQuick™	https://www.systembio.com/micrna-research/exoquick-exosomes/ordering
Exosome Isolation from Plasma	ExoQuick™ Plasma prep and Exosome precipitation kit	https://www.systembio.com/micrna-research/exoquick-exosomes/ordering
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/micrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck™ Assays	https://www.systembio.com/micrna-research/exosome-antibody-arrays
ELISA	ExoELISA™ Kits	https://www.systembio.com/micrna-research/exosome-antibody/elisas
Quantification of Exosomes		
Quantification of exosomes	EXOCET™ Assays	https://www.systembio.com/micrna-research/exosome-antibody/exocet-assay
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir™ kits	https://www.systembio.com/micrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

1. Nanosight

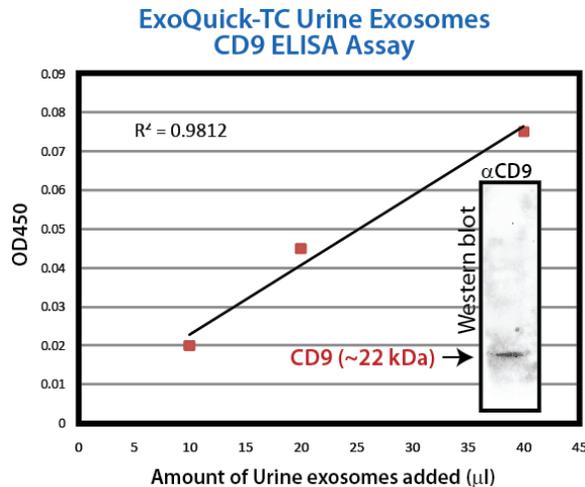
The NanoSight LM10 instrument is based on a conventional optical microscope and uses a laser light source to illuminate nano-scale particles within a 0.3 ml sample introduced to the viewing unit with a disposable syringe. Enhanced by a near perfect black background, particles appear individually as point-scatterers moving under Brownian motion. The image analysis Nanoparticle Tracking Analysis (NTA) software suite allows users to automatically track and size nanoparticles on an individual basis. Results are displayed as a frequency size distribution graph.

For the NanoSight analysis, 2ml of ExoQuick-TC were combined with 10ml of conditioned media from Human HT1080 lung sarcoma cells or Human embryonic kidney (HEK293) cells. 5ml of normal human urine was combined with 2.5 ml of ExoQuick-TC. All samples were incubated overnight at 4°C for exosome precipitation. The exosomes were resuspended in 1ml of PBS and visualized on the NanoSight LM10 instrument (The HT1080 culture media were diluted 1:40 and the urine sample diluted to 1:50 prior to analysis). HT1080 culture media analysis showed that ExoQuick-TC isolated 133nm (peak) exosomes with a recovery of 1.74×10^9 particles/ml. The HEK293 showed 137nm exosomes with a recovery of 3.33×10^8 particles/ml. Normal human urine showed 107nm exosomes with a recovery of 4.8×10^9 particles/ml.



2. Urine exosome protein marker analysis

Ten milliliters of normal human urine was combined with 2ml ExoQuick-TC to precipitate urine exosomes. The exosome pellet was resuspended in 175 μ l buffer and increasing amounts of the exosome suspension was loaded onto an ELISA-ready plate. The CD9 protein was detected using SBI's rabbit anti-CD9 primary antibody and SBI's HRP-conjugated secondary goat anti-rabbit antibody. The size of urine CD9 proteins was determined using Western blot analysis with the same set of antibodies.



Troubleshooting

I don't see a pellet after centrifuging my sample	Scale up the volume of tissue culture media to precipitate more exosomes.
The exosome pellet is difficult to resuspend in buffer	This can be caused by salt within the ExoQuick-TC and can occur after freezing exosome pellets. Try adding slightly more PBS to the pellet. Alternatively, you can use 0.5x PBS or water, and allow the pellet to sit at room temperature for 5-10 minutes before resuspending.

Licensing and Warranty Statement

Limited Use License

Use of the ExoQuick-TC™ Exosome Precipitation Solution (*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.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

- The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.
- The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.
- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

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.

SBI’s liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI’s liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

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