ExoQuick-TC™ Exosome Precipitation Solution

Item Catalog # Reactions
ExoQuick-TC exosome precipitation solution (50 ml) EXOTC50A-1 50 reactions
The ExoQuick-TC™ kits are shipped at room temperature or on blue ice and should be stored at +4°C upon receipt. Properly stored kits are stable for 1 year from the date received. The reaction size is based on using 5 ml of tissue culture media or urine for exosome isolation. Examples of precipitating exosomes from various Biofluids can be seen in the Table below. For Best recovery for both RNA and Protein analysis, we recommend starting with 10 ml sample

Biofluid	Sample volume	ExoQuick-TC volume
Urine	5 ml	1 ml
Spinal fluid	5 ml	1 ml
Culture media	5 ml	1 ml
For best RNA and Protein recovery (10ml sample)		
Urine	10 ml	2 ml
Spinal fluid	10 ml	2 ml
Culture media	10 ml	2 ml

List of components

Item	Catalog #	Reactions
ExoQuick-TC exosome precipitation solution (50 ml)	EXOTC50A-1	50 reactions

ExoQuick-TC Exosome Precipitation

I. Overview
Exosomes are small 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 and proteins depending upon the tissue from which they are secreted. SBI's ExoQuick-TC exosome precipitation reagent makes microRNA and protein biomarker discoveries simple, reliable and quantitative. Enrich for exosomal microRNAs with ExoQuick-TC™ and accurately profile them using SBI's SeraMir™ qPCR arrays. Downstream protein analysis is also possible with SBI's exosome specific antibodies and ELISA kits.

* No time-consuming ultracentrifugation

* Less expensive than costly antibodies and beads
* More effective than any other method
* Use as little as 5 ml media or urine samples

II. PROTOCOL

A. Exosome Precipitation – 10 ml starting sample Isolate exosomes with ExoQuick-TC

1. Collect biofluid and centrifuge at 3000 × *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 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
6 hours-Overnight	Urine	10 ml	2 ml
6 hours-Overnight	Culture media	10 ml	2 ml

3. Refrigerate overnight (at least 12 hours). The tubes do not need to be rotated during the incubation period
4. Centrifuge ExoQuick-TC/biofluid mixture at 1500 × *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. Aspirate supernatant. Spin down residual ExoQuick-TC solution by centrifugation at 1500 × *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 100µl - 500µl of buffer. **Please see the next section of this protocol to determine the appropriate buffer for protein or RNA analysis.**

B. Using Precipitated Exosomes for RNA Extraction

For RNA extraction, we recommend following the protocol outlined in the SeraMir Kit user manual as shown here (Catalog#: RA800A-1, RA805A-1, RA806A-1, RA810A-1, and RA820A-1).
1. If frozen, thaw culture media or urine sample on ice
2. Combine 10ml sample + 2ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for 6 hours to overnight
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet
7. Add 350 µl **LYSIS Buffer** to exosome pellet and vortex 15 seconds
8. Place at room temperature for 5 minutes (to allow complete lysis) *optional--- add 5* µl *of SeraMir control RNA spike-in (cat#RA805A-1)*
9. Add 200µl of 100% **Ethanol**, vortex 10 seconds
10. Assemble spin column and collection tube
11. Transfer all (600µl) to spin column

12. Centrifuge at 13,000 rpm for 1 minute (check to see that all flowed through, otherwise spin longer)
13. Discard flow-through and place spin column back into collection tube
14. Add 400µl **WASH Buffer**
15. Centrifuge at 13,000 rpm for 1 minute
16. Repeat steps 13 to 15 once again (total of 2 Washes)
17. Discard flow-through and centrifuge at 13,000 rpm for 2 minutes to dry (IMPORTANT !)
18. Discard collection tube and assemble spin column with a fresh, RNase-free 1.5ml elution tube (not provided)
19. Add 30µl **ELUTION Buffer** directly to membrane in spin column
20. Centrifuge at 2,000 rpm for 2 minutes (loads buffer in membrane)
21. Increase speed to 13,000 rpm and centrifuge for 1 minute (elutes exoRNAs)
22. You should have recovered 30-40µl exosome RNA

The yield of RNA from isolated exosomes is different depending on the starting biofluid or the type of cells that were grown in culture. Different cell types secrete varying levels of exosomes.

C. Using Precipitated Exosomes for Protein Extraction ELISA analysis

SBI offers three ELISA kits (Catalog#: ExoELISA-63, ExoELISA-9, ExoELISA-81) for fast and quantitative analysis of wellcharacterized exosomal protein markers**: CD63, CD9** and **CD81**.
1. If frozen, thaw culture media or urine sample on ice
2. Combine 10ml sample + 2ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for overnight (at least 12 hours)
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet
7. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
8. Add 200 µl **Exosome Binding buffer** to exosome pellet and vortex 15 seconds
9. Place at room temperature for 5 minutes (to allow complete lysis)
10. Exosome protein is now ready for immobilization onto micro-titer plate.

Please refer to the ExoELISA manual for the complete protocol.

Western blot analysis
For Western blotting analysis, we recommend resuspending the exosome pellet in **1XRIPA buffer**1 with the appropriate protease inhibitor cocktail. SBI offers a Western blot antibody detection kit (Catalog# ExoAB4-1) which includes four exosomal marker antibodies: **CD63, CD9, CD81, HSP70** and a Goat anti-Rabbit IgG HRP conjugated secondary antibody specifically tested for use in exosomal protein analysis.
1. If frozen, thaw culture media or urine sample on ice
2. Combine 10 ml sample + 2 ml **ExoQuick-TC**
3. Mix well by inversion three times
4. Place at 4ºC for overnight (at least 12 hours)
5. Centrifuge at 1500 × *g* for 30 minutes
6. Remove supernatant, keep exosome pellet

7. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
8. Add 200 µl **RIPA buffer1** to exosome pellet and vortex 15 seconds
9. Place at room temperature for 5 minutes (to allow complete lysis)
10. Add **Laemmli buffer**2 (with Beta-mercaptoethanol) and heat at 95ºC for 5 minutes.
11. Chilled on ice for 5 minutes before loading onto gel
12. Perform standard SDS-PAGE electrophoresis and Western transfer onto PVDF membrane
13. Block with 5% dry milk in Tris Buffered Saline + 0.05% Tween (TBS-T) for 1 hour
14. Incubate blot overnight at 4°C with SBI's exosome specific antibody (e.g. CD9) at 1:1000 dilution (5% dry milk in TBS-T)
15. Wash 3X with TBS-T
16. Incubate one hour at room temperature with SBI's Goat anti-Rabbit-HRP antibody at 1:20,000 dilution (5% dry milk in TBS-T)
17. Wash 3X with TBS-T
18. Incubate blot with chemi-luminescence substrate and visualize on film or other imaging equipment

1 1X **RIPA buffer** contains:

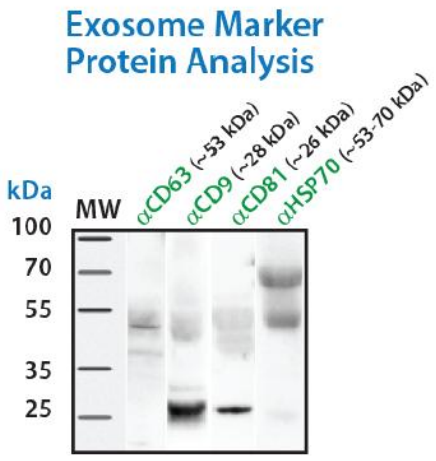
- 25mM Tris-HCl pH 7.6
- 150mM NaCl
- 1% NP-40
- 1% sodium deoxycholate
- 0.1% SDS

2 2X **Laemmli buffer** contains:

ExoQuick-TC™ Exosome Precipitation Solution Cat. # EXOTCxxA-1

- 4% SDS
- 20% glycerol
- 10% 2-mercaptoethanol
- 0.004% bromphenol blue
- 0.125 M Tris-HCl pH 6.8

III. Sample data and applications

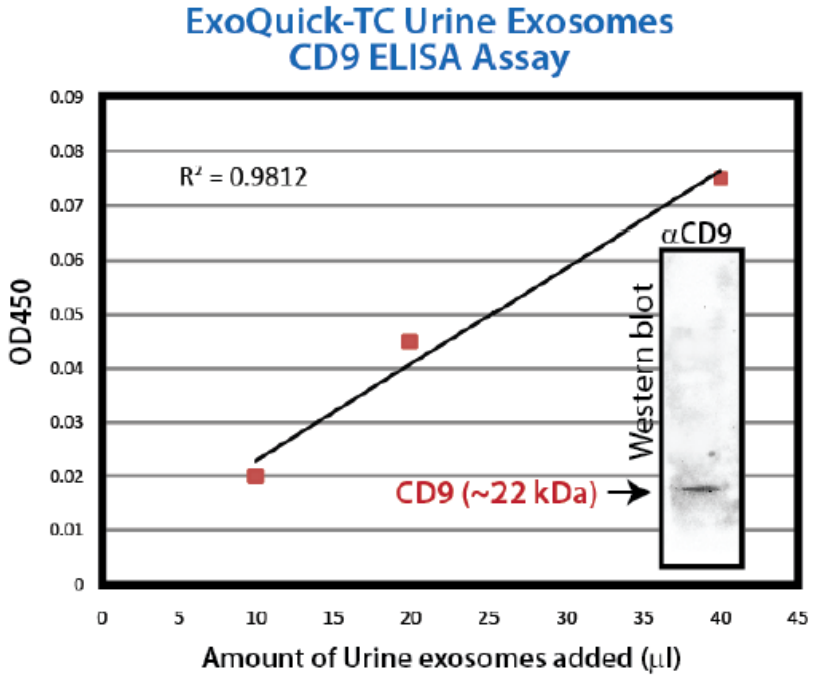


A. NanoSight

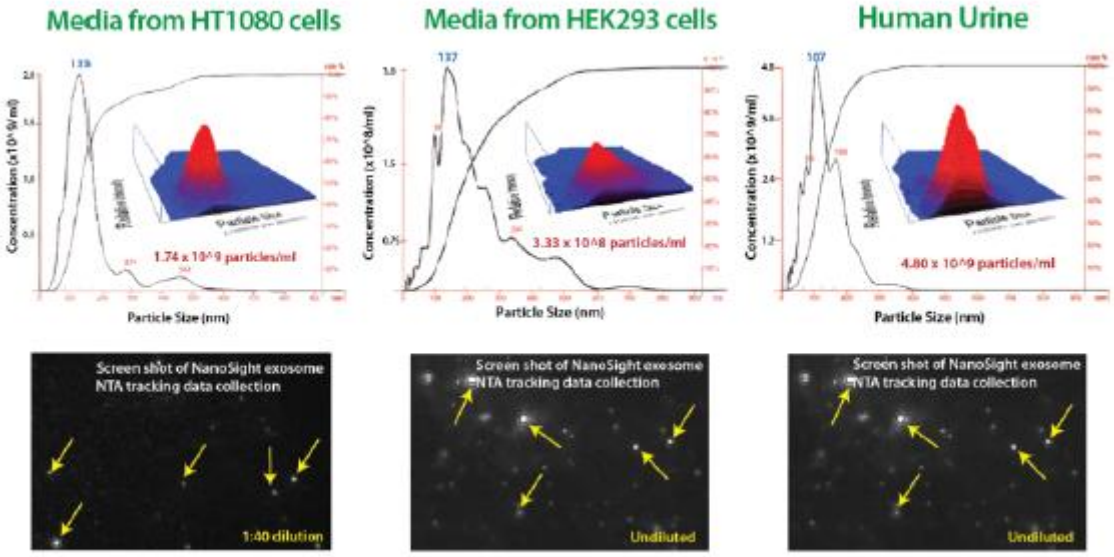
The NanoSight LM10 instrument is based on a conventional optical microscope and uses a laser light source to illuminate nanoscale particles within a 0.3 ml sample introduced to the viewing unit with a disposable syringe. Enhanced by a near perfect black background, particles appear individually as point-scatterers 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 4ºC 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 X109 particles/ml. The HEK293 showed 137nm exosomes with a recovery of 3.33X108 particles/ml. Normal human urine showed 107nm exosomes with a recovery of 4.8X109 particles/ml. For more information on using the NanoSight instrument for exosome analysis, visit: http://www.nanosight.com.

B. Urine Exosome Marker Protein Analysis



Ten milliliters of normal human urine was combined with 2ml ExoQuick-TC to precipitate urine exosomes. The exosome pellet was resuspended 175 □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 (see inset).



C. Activity Assays: Track Exosomes using Cyto-Tracers

SBI has created a line of lentivector-based Cyto-Tracers™ that utilize GFP-fusion proteins to mark cellular compartments, organelles, vesicles and structures to enable more long-term and more in-depth experimentation. The Cyto-Tracers can be used in transfections as well as packaged into virus to create stable GFP tracer cell lines in primary cells, tumor cell lines and stem cells.

The Tetraspanin CD63 protein is a common biomarker for exosomes. With the pCT-CD63-GFP construct you can make you cells of interest secrete exosomes that glow green for downstream functional delivery studies (Cat. # CYTO120-PA-1).