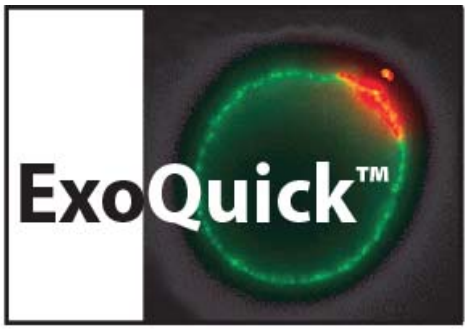


ExoQuick™ Exosome Precipitation Solution

Cat. # EXOQ5A-1
Cat. # EXOQ20A-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.

(ver. 2-2010-07-02)

Contents

List of Components

Item	Catalog #	Reactions
ExoQuick exosome precipitation solution (20 ml)	EXOQ20A-1	300 reactions
ExoQuick exosome precipitation solution (5 ml)	EXOQ5A-1	75 reactions

The ExoQuick™ 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 250 µl serum for exosome isolation. Examples of precipitating exosomes from various bio-fluids can be seen in the Table below.

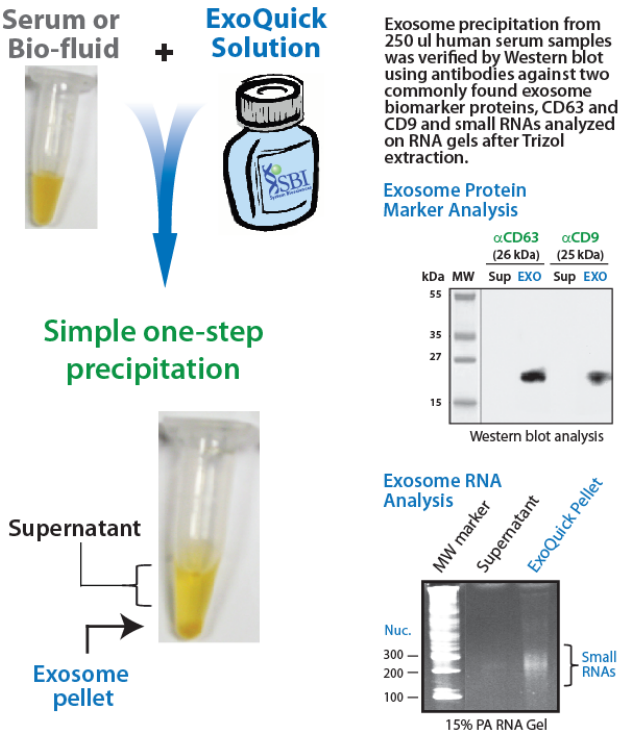
Bio-fluid	Sample volume	ExoQuick volume
Serum	250 µl	63 µl
Ascites fluid	250 µl	63 µl
Urine	1 ml	1 ml
Spinal fluid	1 ml	1 ml
Culture media	1 ml	1 ml

ExoQuick Exosome Precipitation

A. Overview

Exosomes are 40 –100 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 QuantiMir™ 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



B. Protocol

Isolate exosomes with ExoQuick

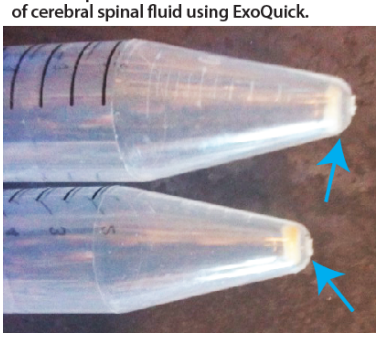
1. Collect bio-fluid and centrifuge at 3000 × *g* for 15 minutes to remove cells and cell debris. Supernatant may be filtered through a 0.45 μm PVDF filter to further eliminate cellular debris. Please note that filtration may decrease the amount of exosomes in the supernatant.
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick Exosome Precipitation Solution to the bio-fluid. Some examples are shown in the Table below. Mix well by inverting or flicking the tube.

Bio-fluid	Sample volume	ExoQuick volume
Serum*	250 µl	63 µl
Ascites fluid	250 µl	63 µl
Urine	1 ml	1 ml
Spinal fluid	1 ml	1 ml
Culture media	1 ml	1 ml

*We do not recommend precipitating exosomes from plasma, as the resulting pellet is difficult to resuspend due to precipitated fibrin and other fibrinogens.

3. Refrigerate overnight (at least 12 hours). The tubes do not need to be rotated during the incubation period.
4. Centrifuge ExoQuick/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 vessel.

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



5. Aspirate supernatant. Spin down residual ExoQuick 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 1/10 of original volume using sterile or nuclease-free water. If the pellet is difficult to resuspend, add slightly more water to the pellet to further dilute the salt.
7. Aliquot in cryogenic vials and store at -70°C until ready for use.

Using Precipitated Exosomes for RNA Extraction

We recommend resuspending the exosome pellet first, and then using TRIzol-LS (Invitrogen, Cat. #10296028) according to the manufacturer's instructions for isolation of RNA from precipitated exosomes.

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. For serum, the level of RNA isolated from 250 µl is usually in the ng/ µl range and can be measured using a GeneQuant II (Agilent Technologies), or can be easily detected on an agarose gel with ethidium bromide.

Using Precipitated Exosomes for Protein Extraction

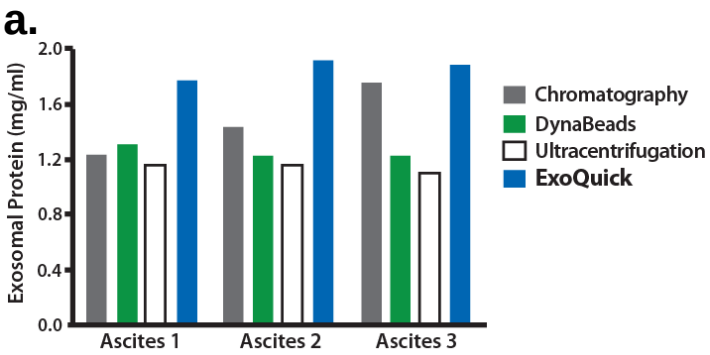
For protein applications, we recommend running a standard protein assay on the resuspended exosome pellet. For SDS-PAGE and Western blotting, 5-10 µg/ well is sufficient for detection of most exosome proteins.

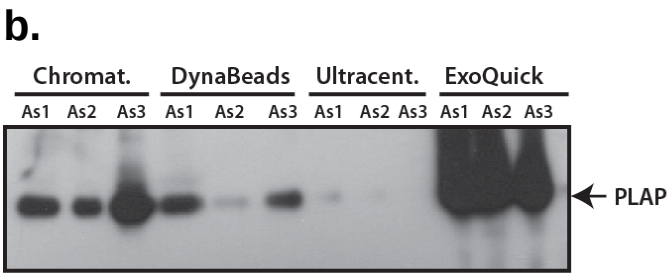
The exact yield of protein from isolated exosomes is different depending on the starting biofluid or the type of cells that were grown in culture. Different cell types secrete varying levels of exosomes, and not all exosomes express the same proteins. A good reference for determining marker proteins for your cell type can be found at:

http://exocarta.ludwig.edu.au/browse

C. Example Data and Applications

1. Protein Yield from Exosomes precipitated with ExoQuick versus other Extraction Methods



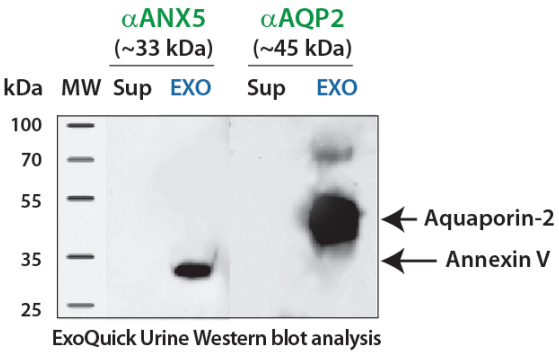


Data courtesy of Dr. Douglas Taylor, Univ. Lousiville, KY.

a. The quantity of protein was determined by the Bradford microassay method (Bio-Rad Laboratories) using BSA as a standard.

b. Proteins from each exosome isolate were standardized to the original sample volume and equal volumes were applied per lane of a 12.5% SDS-PAGE gel. Western immunoblotting was performed to analyze the presence of the specific marker protein, placental alkaline phosphatase (PLAP). The SDS-PAGE gel was transferred to a nitrocellulose membrane, the membrane blocked for 1 hour at room temperature with non-fat dried milk, and probed overnight at 4°C with primary antibody. The bound immune complexes were visualized by enhanced chemiluminescence (ECL, Amersham Life Sciences) and quantitated by densitometry (Un-Scan-it Software, Silk Scientific Corp).

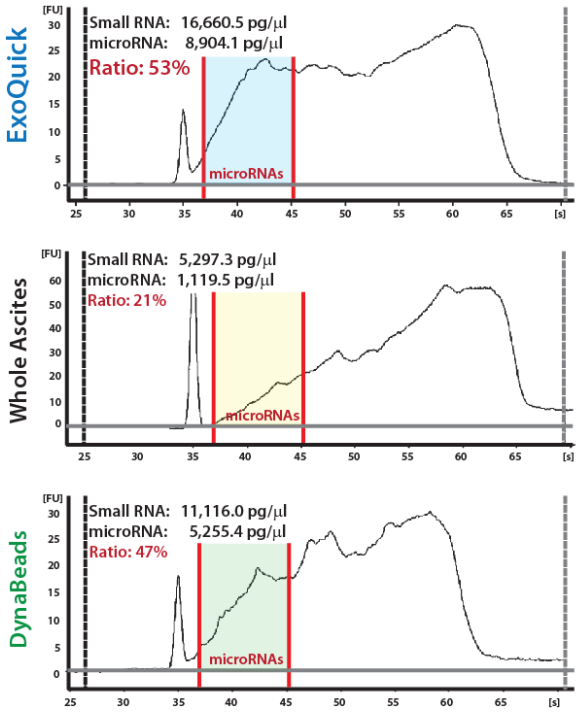
2. Urine Exosome Marker Protein Analysis



ExoQuick Urine Western blot analysis

Human urine samples (1 ml) were treated with 1 ml ExoQuick. Exosome pellets recovered were resuspended in 35µl PBS and the supernatants used as controls. Equal volumes (10µl) of urine supernatant and exosome pellets were separated on 4–15% gradient PAGE gels (Bio-Rad). Standard Western blot procedures with antibodies to Annexin V and Aquaporin-2 (Abcam, Inc.) were used to detect urine exosomal protein markers.

3. MicroRNA Yield from Exosomes precipitated with ExoQuick versus other Extraction Methods



Agilent Bioanalyzer data courtesy of Dr. Douglas Taylor, Univ. Lousiville, KY.

The RNA quality and yield was accessed using a GeneQuant II. Small RNAs were analyzed with the Agilent 2100 Bioanalyzer Lab-on-a-Chip instrument system (Agilent Technologies), using the Agilent Small RNA chip and reagent kit. Approximately 100ng of isolated total RNA in 1µl was applied to each run. The manufacturer's recommended protocol was strictly followed to obtain Bioanalyzer profiles for the size range 6 to 150 nucleotides (nt). The profiles were calibrated for size (nt) using the small RNA ladder supplied with the kit, containing markers of 20, 40, 60, 80, and 150 nt in size, as reference. The instrument software quantitated the peak area between 0 and 150 nt as small RNA region, the area within 10 to 40 nt as microRNA region, and provides percentages of miRNA detected for each sample.

4. 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).