ExoQuick™
Exosome Precipitation Solution

Cat# EXOQ5A-1

Cat# EXOQ20A-1

User Manual

Store kit at Room Temperature or (25°C) or 4°C on receipt

Version 10
1/30/2017

Product Description

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

List of Components

Item	Catalog #	Volume	Reactions
ExoQuick™ Exosome Precipitation Solution	EXOQ20A-1	20 ml	300 reactions
ExoQuick™ Exosome Precipitation Solution	EXOQ5A-1	5 ml	75 reactions

Storage

The ExoQuick kits are shipped at room temperature, blue ice or dry ice and should be stored at +4°C or room temperature (+25°C) upon receipt. Properly stored kits are stable for 1 year from the date received.

General Information

The reaction size is based on using 250 µl of serum for exosome isolation. Examples of precipitating exosomes from various biofluids can be seen in the Table below. These volumes can be scaled up or down accordingly. We recommend a minimum starting sample volume of at least 100 µl.

Bio-fluid	Sample volume	ExoQuick volume
Serum	250 µl	63 µl
Ascites fluid	250 µl	63 µl

To isolate exosomes **from tissue culture media or urine**, we recommend using the **ExoQuick™-TC** reagent (cat# EXOTC10A-1 or EXOTC50A-1) which is a distinct formulation from the original ExoQuick reagent detailed in this manual.

To isolate exosomes from **plasma**, we recommend using the **ExoQuick™ Plasma Prep and Exosome Precipitation Kit** (Cat# EXOQ5TM-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.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Protocol: ExoQuick™

1. Collect biofluid (e.g. serum or ascites fluid) and centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.

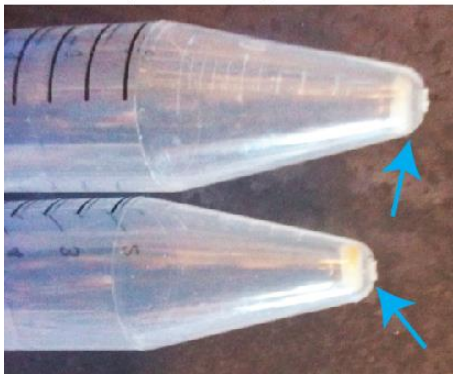
2. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick Exosome Precipitation Solution to the bio-fluid. Some examples are shown in the Table below. Mix well by inverting or flicking the tube.

Incubation Time	Bio-fluid	Sample volume	ExoQuick volume
30 minutes	Serum	250 µl	63 µl
Overnight	Ascites fluid	250 µl	63 µl

3. Refrigerate overnight (at least 12 hours) for ascites fluid or 30 minutes for serum at +4°C. The tubes should not be rotated or mixed during the incubation period and should remain upright.

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 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.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

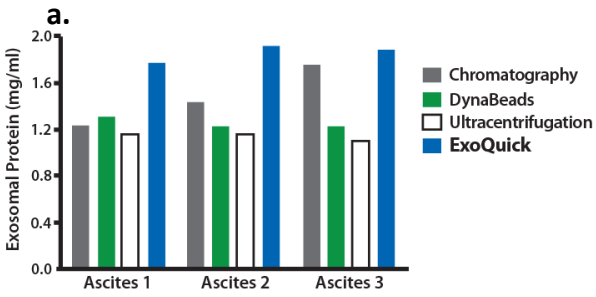
North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

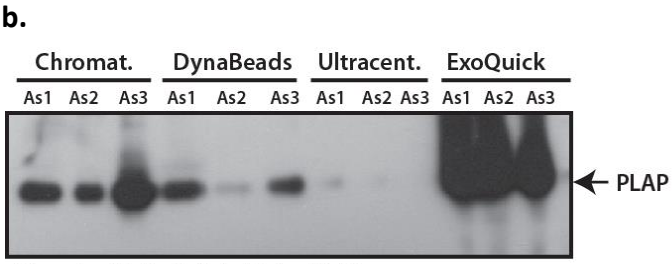
Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Example Data and Applications

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



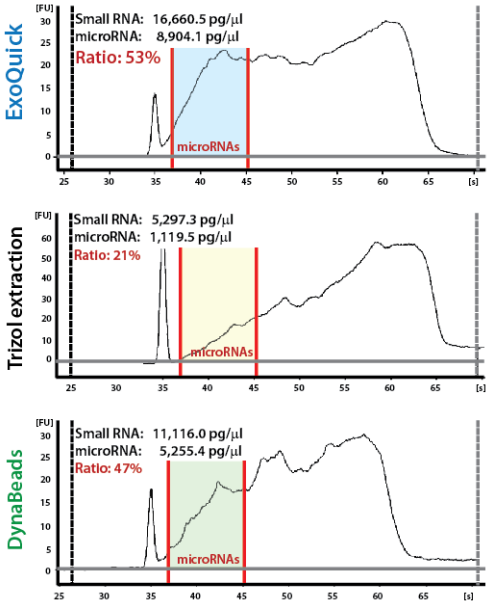
a. The quantity of protein was determined by the Bradford microassay method (Bio-Rad Laboratories) using BSA as a standard.



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

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. 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.

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Troubleshooting

I don't see a pellet after centrifuging my sample	Scale up the volume of biofluid to precipitate more exosomes.
I have precious samples and cannot reach the minimum input volume of 250 µl.	Depending on downstream application, we have demonstrated it is possible to isolate exosomes from as low as 25 µl of starting material by diluting your sample up to 250 µl with PBS and then adding ExoQuick™ in the appropriate ratio (63 µl). **Note**: Although a pellet may be not visible, it is possible that exosomes were in fact isolated. Further downstream analysis is required.
I have a pellet that is not dissolving or that is difficult to resuspend.	If you have started with plasma, please follow the protocol for using thrombin to dissolve the fibrin within the sample before using ExoQuick. ExoQuick formulation contains some salt and occasionally the salt precipitates when the sample is spun down. This can create a pellet that is difficult to resuspend. It is important that you resuspend the pellet in a minimum of >1/10 the original sample volume. We first recommend adding more PBS to the pellet to try to dissolve it, and incubate this with your sample at RT for ~5 min. If the pellet is still difficult to resuspend, spin it down, then try using >1/10 the original volume of 0.5x PBS or ddH_2O. You can also let the pellet sit with the PBS or water at room temperature for 5-10 minutes and then gently try to resuspend the pellet using gentle agitation (pipet up and down, gentle vortex).