ExoQuick® ULTRA EV Isolation Kit for Serum and Plasma

Cat # EQULTRA-20A-1

User Manual

Storage: Please see individual components

Version 2
6/29/2018

Contents

Product Description

Isolation of extracellular vesicles (EVs) from biofluids such as serum and plasma has been a challenging and sometimes frustrating roadblock to getting to what matters the most – understanding the biology of EVs. The presence of carryover molecules (e.g. proteins) in these biofluids often masks what we are able to detect and becomes a formidable challenge to successful outcomes. Currently available methods to isolate EVs face formidable trade-offs in terms of yield, ease of use, purity, and price – a practical challenge in itself to researchers who care about getting to the "what" as opposed to the "how".

To address these issues, SBI is proud to introduce ExoQuick™ ULTRA EV Isolation Kit for Serum and Plasma (Cat #EQULTRA-20A-1) – an innovative kit drawing upon our expertise in the exosome isolation space. It is the first-in-class kit designed to avoid the trade-offs faced with other EV isolation methods. Now researchers have the power to singularly focus on the challenges of EV biology without worrying about bottlenecks of EV isolation.

The kits come with SBI's proven ExoQuick™ EV isolation reagent as well as our convenient, pre-packed bipartate resin columns good for 20 reactions*. Start from 250 µl of serum or plasma and in less than 20 min of total hands-on time, researchers have high-quality EVs for downstream applications such as such as western blotting, mass spectrometry, NGS sequencing, exosome labeling, and *in vivo/ex vivo* exosome delivery.

***Serum/Plasma: 1 reaction is defined as 250 ul of serum/plasma precipitated using ExoQuick**

List of Components

Component	Qty/Volume	Storage Temperature
ExoQuick	2 ml	RT
Purification column	20 columns	4°C
Collection tubes	20 tubes	RT
2 ml Eppendorf tubes	20 tubes	RT
Buffer A	5 ml	4°C
Buffer B	30 ml	4°C

Storage

The Kit is shipped on blue Ice and the components should be stored at recommended temperatures as stated above. Properly stored kits are stable for 12 months from the date received.

General Information

NOTE: For plasma samples, addition of thrombin (Cat #TMEXO-1) to generate a serum-like fraction is an optional step, especially for sensitive applications such as mass spectrometry, where additional proteins added to the sample may affect detection of certain low abundance proteins.

Protocol

A. ExoQuick Isolation

1. Collect the biofluid and centrifuge at 3,000 × g for 15 minutes to remove cellular debris.

2. Transfer the supernatant to a new tube.

! **OPTIONAL: If additional debris remains detectable, centrifuge the supernatant for additional 10 minutes at 12,000 x g and transfer the supernatant to a new tube.**

3. Add the appropriate volume of ExoQuick to the clarified biofluid as shown in the table.

Biofluid	Sample Volume	ExoQuick Volume	Incubation Time
Serum	250 µl	67 µl	30 min at 4°C
Plasma	250 µl	67 µl	30 min at 4°C

4. Mix well by inverting or flicking the tube, and incubate on ice for 30 minutes. The tubes do not need to be rotated during the incubation period.

5. Centrifuge the ExoQuick/biofluid mixture at 3,000 × g for 10 minutes. Centrifugation may be performed at either room temperature or 4°C with similar results. After centrifugation, the EVs may appear as a beige or white pellet at the bottom of the tube.

6. Carefully aspirate off the supernatant. Spin down any residual ExoQuick solution and remove all traces of fluid by aspiration, taking great care not to disturb the precipitated EVs in the pellet.

7. Resuspend the pellet in 200 µl of Buffer B.

8. Measure and record sample protein concentration.

B. Purification of Isolated EVs

1. Add 200 µl of Buffer A to resuspended EVs

2. Take out Purification column, loosen screw cap and snap off the bottom closure. Place the column into a collection tube.

! **NOTE: Save the bottom closure for steps 7-9.**

3. Centrifuge at 1,000 x g for 30 seconds to remove the storage buffer.

4. Discard the flow-through and place the column back into the collection tube.

5. To wash the column, remove the cap and apply 500 µl of Buffer B on top of the resin and centrifuge at 1,000 x g for 30 seconds. Discard the flow through.

! **NOTE: Save the cap for steps 7-9.**

6. Repeat steps 4 – 5 one more time to wash the column.

7. Plug the bottom of the column with the bottom closure. Apply 100 µl of Buffer B on top of the resin to prep it for sample loading.

8. Add the entire content from step 1 (or up to volume equivalent of 4 mg of total protein) to the resin. Place the screw cap on the top of the column.

9. Mix at room temperature (RT) on a rotating shaker for no more than 5 minutes.

C. Sample Elution

! **CAUTION: Sample will start to elute as soon as the bottom closure is removed. Please make sure 2 ml Eppendorf tubes are ready to receive eluate to minimize sample loss.**

1. Loosen the screw cap and remove the bottom closure, and immediately transfer to 2 ml Eppendorf tube.

2. Centrifuge at 1,000 x g for 30 seconds to obtain purified EVs.

3. Discard the column.

Example Data and Applications

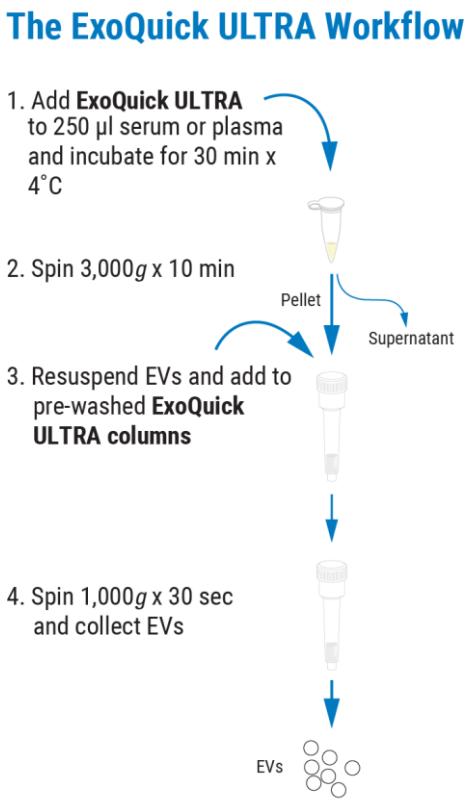

Figure 1. Workflow for ExoQuick ULTRA EV Isolation Kit. Highly purified EVs from 250 µl of serum or plasma can be obtained in less than 20 minutes of hands-on time, with the highest EV yields per 1 ml of input.

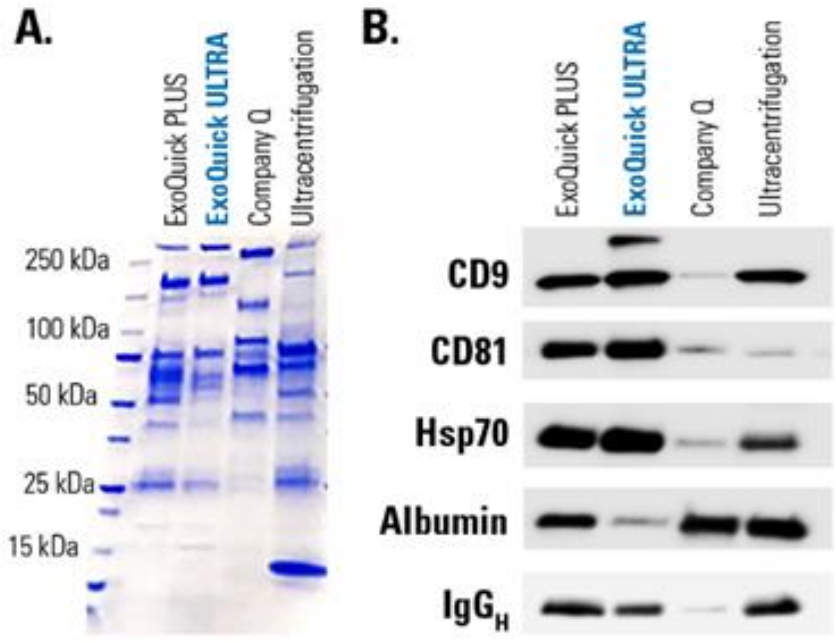

Figure 2. ExoQuick ULTRA delivers higher yields and purity than competing EV isolation methods (A) Coomassie blue-stained protein gel comparing the protein content of exosome preps isolated using different methods shows only a few, defined protein bands in the ExoQuick ULTRA lane compared to the other methods. **(B)** Western blotting shows ExoQuick ULTRA prep contains the highest levels of exosome-specific markers CD9, CD81, and Hsp70 and lowest levels of the carryover proteins albumin and IgG_H. Each lane was loaded with 7 µg of total protein as measured using Qubit fluorimetric protein assay.

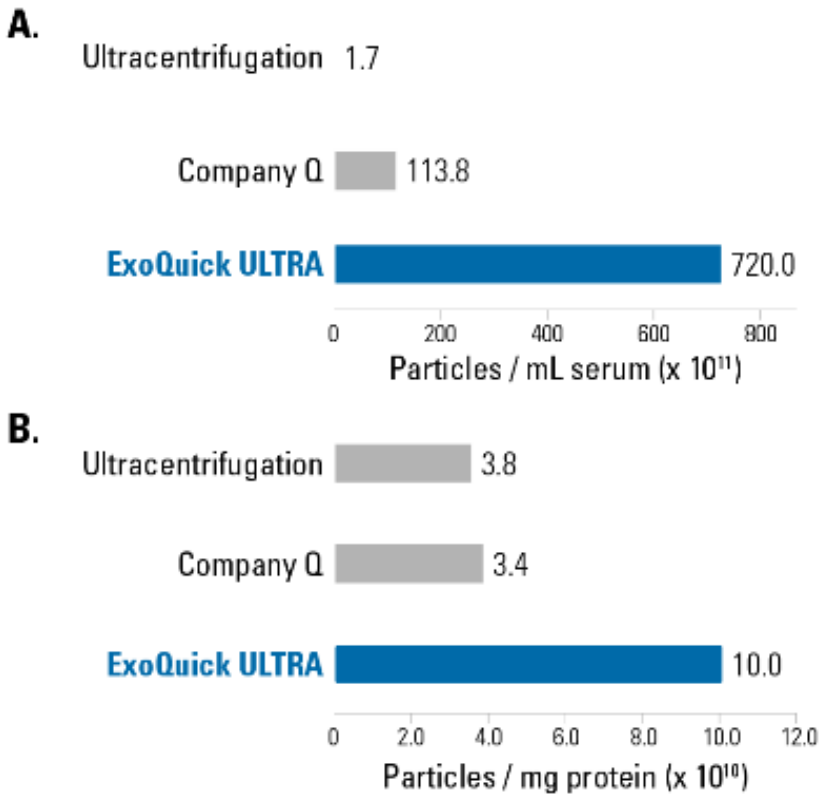

Figure 3. Fluorescent nanoparticle tracking analysis (fNTA) demonstrates the high EV yields delivered by ExoQuick ULTRA. Comparison of different isolation methods of EV yields by **A)** both volume of input serum (per mL) and **B)** amount of input serum protein (per mg as measured by fluorometric Qubit protein assay). Particle number was measured using fNTA, a technique which specifically labels and detects EVs using the fluorescence mode in a nanoparticle tracking instrument.

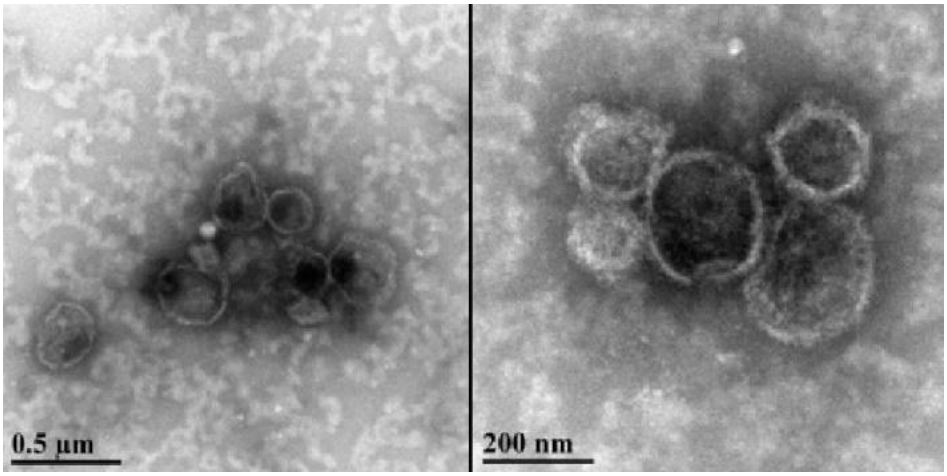

Figure 4. EVs isolated using ExoQuick ULTRA display typical EV morphology. Transmission electron micrographs (TEM) of EVs isolated from human serum using ExoQuick ULTRA. The same sample is shown at two different magnifications. Multiple vesicles with typical EV morphology can be seen in each image.

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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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.

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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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