
ExoQuick PLUS™

Exosome Purification Kit for Serum & Plasma

Cat# EQPL10A-1

User Manual

Store kit at +4°C

Version 2
2/22/2017

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Product Description

ExoQuick PLUS™ is the latest solution for addressing protein carryover from serum and plasma-derived exosomal preparations. It represents an evolution of traditional precipitation-based exosomal preps to address needs for downstream applications where exosomal purity is a concern (e.g. mass spectrometry, exosome labeling, *in vivo/ex vivo* exosome delivery).

The kit comes with ExoQuick as well as our convenient, pre-packed Microsphere beads good for 10 reactions*. With less than 10 min of total hands-on time from precipitated exosomes to purified exosomes, ExoQuick PLUS provides a simple, yet effective solution for obtaining high-purity, intact exosomes for most downstream applications.

***1 reaction is defined as 500ug of protein from 250uL of serum/plasma precipitated using ExoQuick and resuspended in 250uL of Resuspension Buffer in the kit.**

List of Components

Item	Volume	Storage Temperature
ExoQuick Reagent	2 mL	4°C
ExoQuick PLUS Microsphere Beads	10 units	4°C
Resuspension Buffer	12 mL	4°C

Storage

The ExoQuick PLUS kits are shipped at blue ice and should be **stored** at +4°C. Properly stored kits are stable for 6 months from the date received.

General Information

The reaction size is based on using 250 uL of serum or plasma sample.

To isolate exosomes from **plasma**, we recommend using the **Thrombin Plasma Prep for Exosome Precipitation Reagent** (Cat# TMEXO-1, not included). Plasma contains fibrin which will precipitate along with ExoQuick causing an insoluble pellet to form. The thrombin reagent will help to dissolve the fibrin, thus increasing the yield of exosomes precipitated.

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Protocol for ExoQuick PLUS

1. Collect biofluid and centrifuge at 3000 × g for 15 minutes to remove cellular debris.
2. Transfer supernatant to a sterile tube and add the appropriate volume of ExoQuick 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 volume
30min	Serum	250uL	67uL
30min	Plasma*	250uL	67uL

*Pre-treat the plasma with thrombin (TMEXO-1) to obtain serum-like fraction

3. Incubate on ice for 30 minutes. 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 tube.
5. Carefully aspirate off 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 the exosome pellet in a volume of 250uL- 500uL Resuspension Buffer. Measure and record the protein concentration.
7. Pretreat 1 unit of the beads with 400uL of Resuspension buffer. Shake/vortex the solution for 3 minutes.
8. Spin down the beads at 3000 x g for 3 minutes.
9. Remove supernatant and repeat the wash with Resuspension buffer for a total of two washes (step 7 and 8).
10. Remove all the liquid. To the washed beads, add 500ug protein equivalent of exosomes with enough Resuspension buffer to bring the total reaction volume to 250ul.
Example: 25ul of exosomes (500ug) + 225ul Resuspension buffer added to the washed beads.
11. Mix at room temperature on an inverting shaker for 15 minutes.
12. Spin your sample for 5 minutes at 6000 x g.
13. The supernatant contains purified exosomes.

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Example Data and Applications

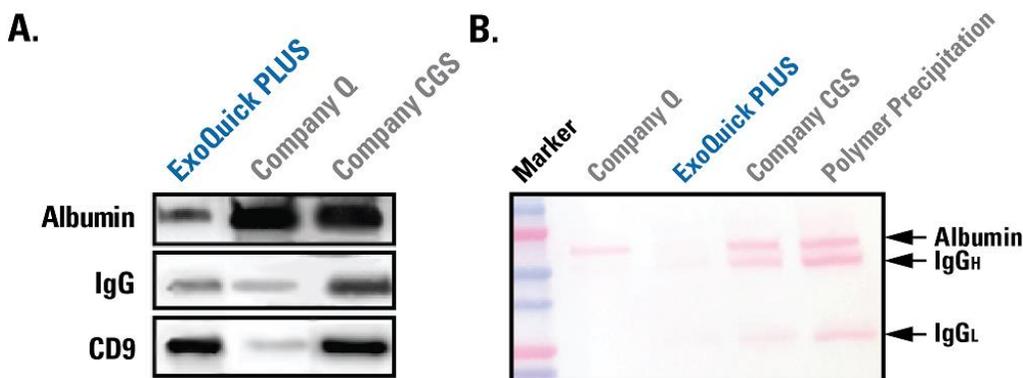


Figure 1. ExoQuick PLUS exosome isolation results in samples with less protein carry-over than competitors' products. For each lane, 2.5 μg of total protein was loaded. **A.** Western blot probing for serum albumin, IgG, and the exosome-specific tetraspanin marker CD9 shows higher CD9:IgG and CD9:albumin ratios in the ExoQuick PLUS lane than in the competitors' lanes. **B.** Ponceau staining to assess total serum protein abundance shows the least amount of carry-over protein in the ExoQuick PLUS lane.

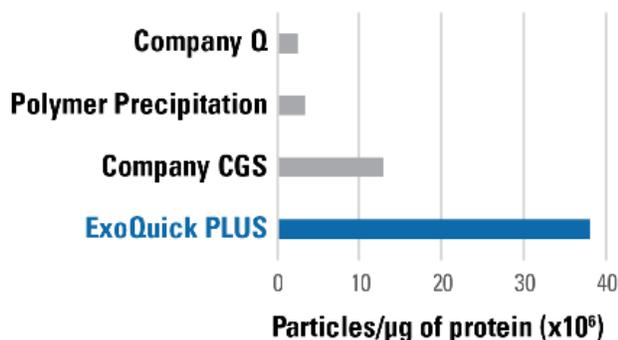


Figure 2. Exosome isolation from serum with ExoQuick PLUS results in a higher particle:protein ratio than competitors' products, indicating less protein carry-over. The particle:protein ratio is a measure of relative protein carry-over, with a higher ratio indicating less protein carry-over. Particle numbers were generated using NanoSight NTA analysis, and carry-over protein concentrations were measured using a BCA assay.

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