ExoQuick-CG™

# ExoQuick-CG™ Exosome Precipitation Solution

**Cat. # EXOCGxxA-1**

***User Manual***

**Store kit at +25° C on receipt**

## Contents



## List of Components

| Item | Catalog # | Reactions |
|---|---|---|
| ExoQuick-CG exosome precipitation solution (50 ml) | EXOCG50A-1 | 30 reactions |

The ExoQuick-CG™ kits are shipped at room temperature or on blue ice and should be stored at +25°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-CG volume |
|---|---|---|
| Urine | 5 ml | 1.6 ml |
| Spinal fluid | 5 ml | 1.6 ml |
| Culture media | 5 ml | 1.6 ml |
| For best RNA and Protein recovery (10 ml sample) | | |
| Urine | 10 ml | 3.3 ml |
| Spinal fluid | 10 ml | 3.3 ml |
| Culture media | 10 ml | 3.3 ml |

# ExoQuick-CG 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-CG exosome precipitation reagent makes microRNA and protein biomarker discoveries simple, reliable and quantitative. Enrich for exosomal microRNAs with ExoQuick-CG™ 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
* Ideal for pre-clinical exosome development

## Manufactured for SBI with cGMP quality by Central Biomedia, Inc.

CENTRAL BIOMEDIA INC

QUALITY STATEMENT

Central Biomedia is registered with the FDA as a Class I Medical Device contract manufacturer, registration number 1932313. Our Quality Systems meet applicable sections of 21 CFR 820, including annual training of all employees on Good Manufacturing Practices.

Kim McCall, Quality Manager

03FEB16
Date

- 21 CFR 820 compliant
- All ingredients sourced from GMP manufacturer
- Reagent mixed under cGMP conditions at GMP facility
- Triple filtered
- Sterility tested
- Works in all biofluids tested

ExoQuick exosome isolation methods are a patented technology. Antes, T. et al. Methods for Microvesicle Isolation and Selective Removal. Patent No.: US 9,005,888 B2

The process of manufacturing of Exo-FBS is a patented method in Patent No.: US 9,005,888 B2.

# PROTOCOL

## A. Exosome Precipitation – 10 ml starting sample

### Isolate exosomes with ExoQuick-CG

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-CG 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-CG volume |
|---|---|---|---|
| **12 hours-Overnight** | **Urine** | **10 ml** | **3.3 ml** |
| **12 hours-Overnight** | **Culture media** | **10 ml** | **3.3 ml** |

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

**Exosome Isolation and Lysis**

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10 ml sample + 3.3 ml **ExoQuick-CG**
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)*

**exoRNA Purification**

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 !)

**exoRNA Elution**

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 well-characterized exosomal protein markers**: CD63, CD9** and **CD81**.

**Exosome Isolation and immobilization**

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10 ml sample + 3.3 ml **ExoQuick-CG**
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. Incubate at 37 ºC temperature for 20 minutes to liberate exosome proteins
10. Centrifuge at 1500 × *g* for 5 minutes to remove all residual precipitation solution
11. Transfer supernatant to new centrifuge tube on ice
12. 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 ExoAb Antibody Sampler Kit (Cat# EXOAB-KIT-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.

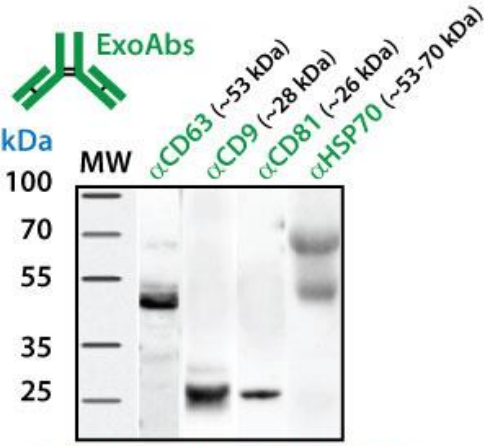
**Exosome Isolation and lysis**

1. If frozen, thaw culture media or urine sample on ice
2. Combine 10 ml sample + 3.3 ml **ExoQuick-CG**
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 buffer**[1] 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

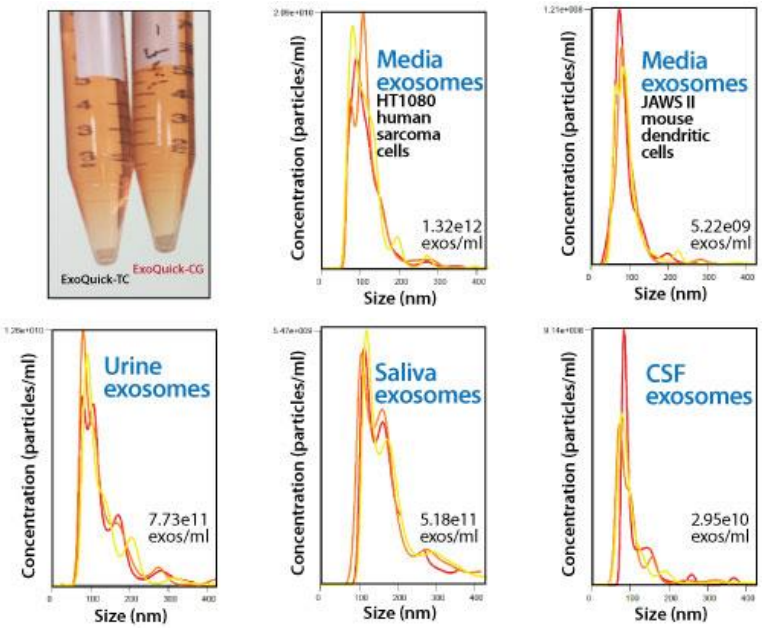
[2] 2X **Laemmli buffer** contains:
- 4% SDS
- 20% glycerol
- 10% 2-mercaptoethanol
- 0.004% bromphenol blue
- 0.125 M Tris-HCl pH 6.8




## 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 nano-scale 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. ExoQuick-CG enables cGMP isolation of exosomes at equivalent rates to ExoQuick-TC.

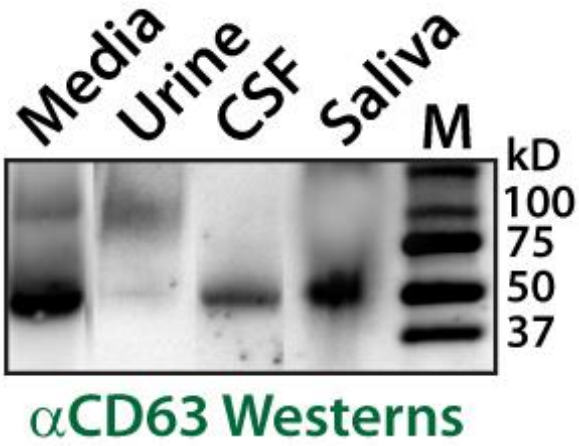



For the NanoSight analysis, 3.3 ml of ExoQuick-CG was combined with 10 ml of conditioned media from Human HT1080 lung sarcoma cells or Mouse JAWS II dendritic cells that had been cultured in Exo-FBS (exosome depleted FBS medium supplement, catalog # EXO-FBS-50A-1). All samples were incubated overnight at 4ºC for exosome precipitation. The exosomes were resuspended in 0.5 ml of PBS and visualized on the NanoSight LM10 instrument.

| Sample type | Starting biofluid volume | ExoQuick-CG added | Exosomes yield (exos/ml) |
|---|---|---|---|
| HT1080 cell media | 10 ml | 3.3 ml | 1.32 x 10^12 |
| JAWS II cell media | 10 ml | 3.3 ml | 5.22 x 10^9 |
| Human Urine | 10 ml | 3.3 ml | 7.73 x 10^11 |
| Human Saliva | 5 ml | 1.6 ml | 5.18 x 10^11 |
| Human CSF | 5 ml | 1.6 ml | 2.95 x 10^10 |

For more information on using the NanoSight instrument for exosome analysis, visit: http://www.nanosight.com.

## B. Exosome Marker Protein Analysis

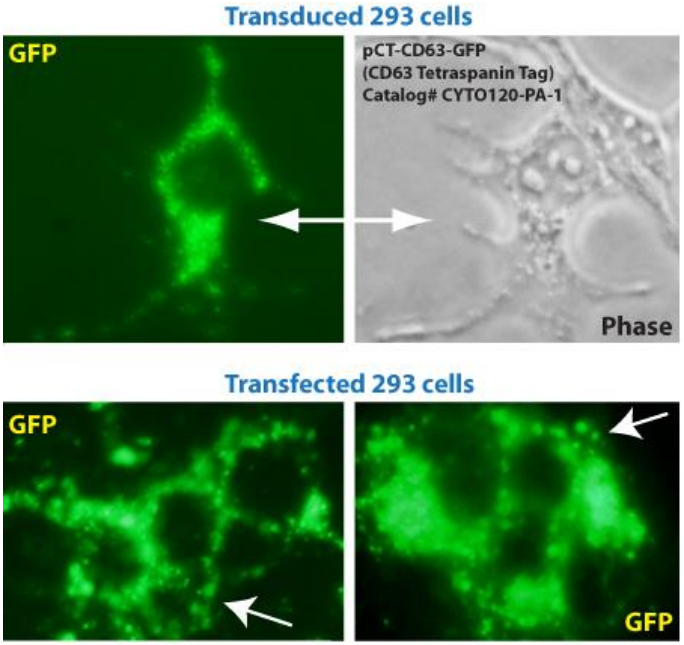



Approximately 10-50 ug of exosomes from the NanoSight studies from above were separated on a denaturing SDS PAGE for Western blot analysis. The CD63 protein was detected using SBI's rabbit anti-CD63 primary antibody and SBI's HRP-conjugated secondary goat anti-rabbit antibody (catalog # EXOAB-CD63A-1).

## 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).




## IV. Citations

**Exosome Isolation for Proteomic Analyses and RNA Profiling**
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Tae Hoon Lee, Esterina D'Asti, Nathalie Magnus, Khalid Al-Nedawi, Brian Meehan and Janusz Rak. Review: Microvesicles as mediators of intercellular communication in cancer—the emerging science of cellular 'debris'. Seminars in Immunopathology DOI: 10.1007/s00281-011-0250-3. (PDF) »

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Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009 Jun;6(3):267-83. Review.

# V. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

**http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

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General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

# VI. Licensing and Warranty Statement

**Limited Use License**

Use of the ExoQuick-CG™ Exosome Precipitation Solution (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

- ExoQuick exosome isolation methods are a patented technology. Antes, T. et al. Methods for Microvesicle Isolation and Selective Removal. Patent No.: US 9,005,888 B2
- The process of manufacturing of Exo-FBS is a patented method in Patent No.: US 9,005,888 B2.

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.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

**Limited Warranty**

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.
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