

Exosome Antibodies & ELISA Kits

Cat #s EXOABxxx, EXOELxxx

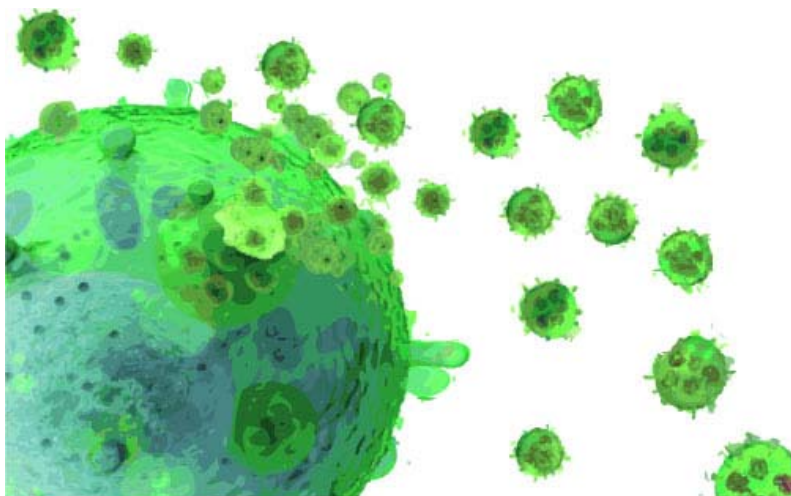
User Manual

See page #2 for product storage conditions

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.

Contents

| | |
|---|----------|
| List of components | 2 |
| Exosome Antibodies | 3 |
| I. Recommended protocol | 3 |
| II. Serum exosome Western data | 4 |
| ExoELISA kit | 5 |
| I. ExoELISA principle | 5 |
| II. Equipment supplied by user | 5 |
| III. Protocol | 5 |
| A. Exosome precipitation with ExoQuick/ExoQuick-TC | 5 |
| B. Exosome isolated with ultracentrifugation method | 6 |
| C. Exosome protein standard | 7 |
| D. ELISA procedures | 8 |
| E. Sample Data | 9 |
| IV. Citations | 9 |
| V. Technical references | 9 |
| VI. Technical Support | 10 |
| VII. Licensing and Warranty Statement | 11 |



List of Components

EXOAB Series

| Exosome Antibodies Components | Amount |
|--|--------|
| Exosome specific primary antibody (CD63,CD9 ,CD81 or Hsp70) | 25 µl |
| Exosome validated secondary antibody (Goat anti-Rabbit HRP) | 5 µl |

The Exosome Antibodies are shipped in blue ice and should be **stored at +4°C** upon receipt. . Properly stored antibodies are stable for 6 months from the date received. They can be place at **-20°C for long term storage**.

EXOEL Series

| ExoELISA kit Components | Amount |
|---|-----------|
| Exosome binding buffer | 20 ml |
| 20X Wash buffer | 18 ml |
| Blocking buffer | 30 ml |
| ExoELISA protein standard* | 400 µl |
| Exosome specific primary antibody (CD63,CD9 or CD81) | 2 x 25 µl |
| Exosome validated secondary antibody (Goat anti-Rabbit HRP) | 10 µl |
| ELISA substrate (Super-sensitive TMB) | 6 ml |
| Stop buffer | 6 ml |
| 96 well ExoELISA plate (12x8 well strips) | 1 plate |

The ExoELISA™ kits are shipped in blue ice and should be **stored at +4°C** upon receipt. Properly stored kits are stable for 6 months from the date received.

* ExoELISA protein standards should be **stored at -20°C** upon receipt. We recommend making single-use aliquots to avoid repeated freeze-thawing.

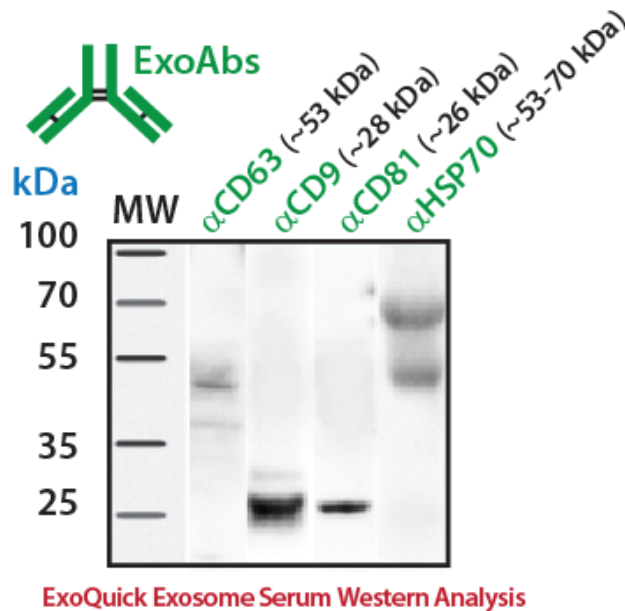
Exosome Antibodies (Cat# EXOAB series)**I. Recommended protocol****-for isolating serum exosomes for Western blotting analysis**

1. If frozen, thaw 250 μ l serum on ice
2. Centrifuge at 1500 \times g for 15 minutes to remove cells and cell debris
3. Transfer sample supernatant to a centrifuge tube
4. Combine 250 μ l sample + 63 μ l **ExoQuick™**
5. Mix well by inversion three times
6. Place at 4°C for at least 30 minutes
7. Centrifuge at 1500 \times g for 5 minutes
8. Remove supernatant, keep exosome pellet
9. Centrifuge at 1500 \times g for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
10. Add 200 μ l **RIPA buffer**¹ (with appropriate protease inhibitor cocktail added) to exosome pellet and vortex 15 seconds
11. Place at room temperature for 5 minutes (to allow complete lysis)
12. Perform standard Bradford protein assay to determine yield.
13. Add **Laemmli buffer**² (with Beta-mercaptoethanol) and heat at 95°C for 5 minutes.
14. Chilled on ice for 5 minutes before loading onto gel
15. Perform standard SDS-PAGE electrophoresis and Western transfer onto PVDF membrane
16. Block with 5% dry milk in Tris Buffered Saline + 0.05% Tween (TBS-T) for 1 hour
17. Incubate blot overnight at 4°C with **Exosome specific primary antibody** (e.g. CD9) at **1:1000 dilution** (5% dry milk in TBS-T)
18. Wash 3X with TBS-T
19. Incubate one hour at room temperature with **Exosome validated secondary antibody** (Goat-Rabbit-HRP) antibody at **1:20,000 dilution** (5% dry milk in TBS-T)
20. Wash 3X with TBS-T
21. 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

II. Serum Exosome Western Data



Western blot analysis of serum exosomes using the recommended protocol. 20 µg of serum exosome proteins were loaded into each lane. All **Exosome specific primary antibody** were used at **1:1,000** dilution and **Exosome validated secondary antibody** were used at **1:20,000** dilution.

ExoELISA™ - (Cat# EXOEL series)

I. ExoELISA principle

1. The ExoELISA kit is designed as a direct Enzyme-Linked ImmunoSorbent Assay (ELISA). The exosome particles and their proteins are directly immobilized onto the wells of the microtiter plate.
2. After binding, wells are coated with a block agent to prevent non-specific binding of the detection antibody.
3. The detection (primary) antibody is added to the wells for binding to specific antigen (e.g. CD63) protein on the exosomes.
4. A Horseradish Peroxidase enzyme linked secondary antibody (Goat anti-Rabbit) is used for signal amplification and to increase assay sensitivity.
5. A colorimetric substrate (Extra-sensitive TMB) is used for the assay read-out. The accumulation of the colored product is proportional to the specific antigen present in each well.
6. The results are quantitated by a microtiter plate reader at 450 nm absorbance.

II. Equipment to be supplied by user

1. Microtiter plate sealing film/cover
2. 37°C incubator
3. Microtiter plate shaker
4. Microtiter plate spectrophotometer with 450 nm absorbance capability
5. Multichannel pipets (recommended)

III. Protocol

A. Exosome precipitation with ExoQuick/ExoQuick-TC

For simple and quick isolation of exosomes from serum, we recommend using the ExoQuick precipitation solution (Catalog# EXOQ5A-1 or EXOQ20A-1) and the ExoQuick-TC for isolation of exosomes from tissue culture media and urine samples (EXOTC10A-1 or EXOTC50A-1).

1. If frozen, thaw sample on ice
2. Centrifuge at 3000 × g for 15 minutes to remove cells and cell debris.
3. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick or ExoQuick-TC.

| Incubation time | Bio-fluid | Sample volume | ExoQuick volume | ExoQuick-TC volume |
|-----------------|---------------|---------------|-----------------|--------------------|
| 30 minutes | Serum | 250 µl | 63 µl | - |
| Overnight | Ascites fluid | 250 µl | 63 µl | - |
| Overnight | Culture Media | 5 ml | - | 1 ml |
| Overnight | Urine | 5 ml | - | 1 ml |
| Overnight | Spinal fluid | 5 ml | - | 1 ml |

4. Mix well by inversion three times
5. Place at 4 °C from 30 minutes to overnight according to table.
6. Centrifuge at 1500 × g for 30 minutes
7. Remove supernatant, keep exosome pellet
8. Centrifuge at 1500 × g for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
9. Add 200 µl **Exosome Binding buffer** to exosome pellet and vortex 15 seconds
10. Incubate at 37 °C temperature for 20 minutes
11. Centrifuge at 1500 × g for 5 minutes to remove all residual precipitation solution
12. Transfer supernatant to new centrifuge tube on ice
13. Exosome protein is now ready for immobilization onto micro-titer plate

Proceed to section - **C. Exosome protein standard curve**

B. Exosome isolated with ultracentrifugation method

1. Performed exosome isolation by the user's preferred ultra-centrifugation protocol
2. Carefully remove all traces of supernatant, keep exosome pellet
3. Resuspend exosome pellet in 200 µl of **Exosome binding buffer** and vortex 15 seconds
4. Incubate at room temperature for 10 minutes and keep on ice.
5. Exosome protein is now ready for immobilization onto micro-titer plate

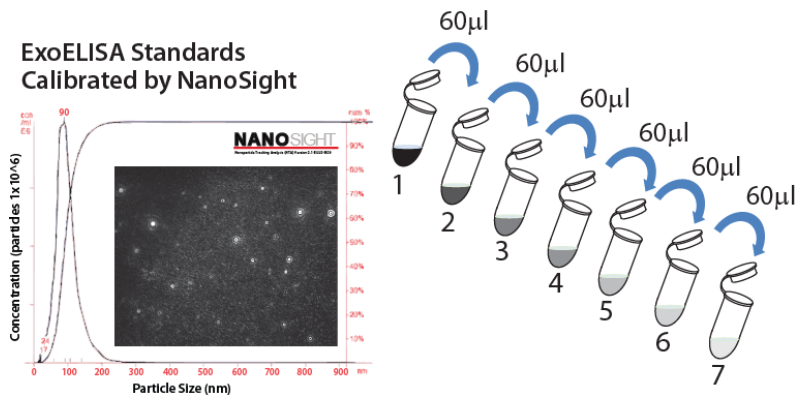
Proceed to section - **C. Exosome protein standard curve**

C. Exosome protein standard curve

A standard curve should be prepared each time the assay is performed

1. Thaw **ExoELISA protein standard** on ice
2. Dilute **ExoELISA protein standard** by performing serial dilutions with **Exosome Binding buffer** in microcentrifuge tubes
3. Suggested dilutions for standard curve:

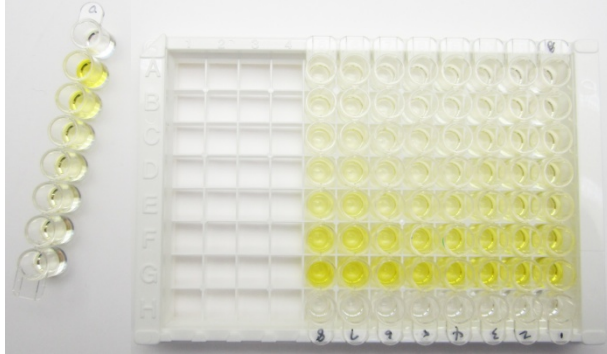
| Tube | # Exosomes | Dilution factor | ExoELISA protein standard | Exosome binding buffer |
|------|-----------------------|-----------------|---------------------------|------------------------|
| 0 | 0 | Blank | - | 60 |
| 1 | 1.35×10^{10} | 1 | 60 μ l | - |
| 2 | 6.75×10^9 | 1:2 | 60 μ l | 60 μ l |
| 3 | 3.37×10^9 | 1:4 | 60 μ l | 60 μ l |
| 4 | 1.68×10^9 | 1:8 | 60 μ l | 60 μ l |
| 5 | 8.44×10^8 | 1:16 | 60 μ l | 60 μ l |
| 6 | 4.21×10^8 | 1:32 | 60 μ l | 60 μ l |
| 7 | 2.10×10^8 | 1:64 | 60 μ l | 60 μ l |



D. ELISA procedures

Before starting

1. The ExoELISA micro-titer plate is provided in a convenient 12 well X 8 strip format. We recommend using at least one strip for the standard curve and additional strips depending on the number of samples tested. Unused 8-well strips can be removed and stored at room temperature for later use.

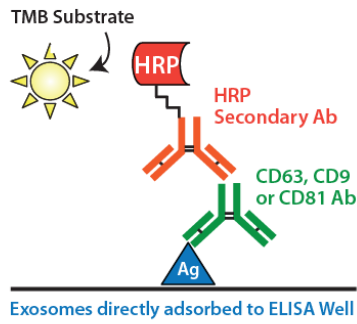
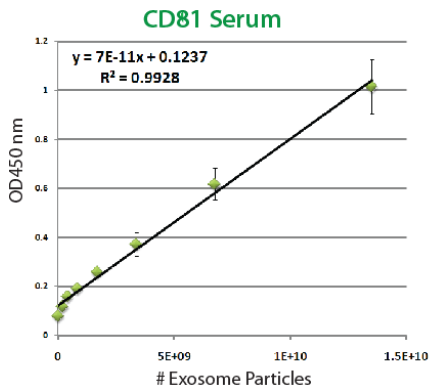
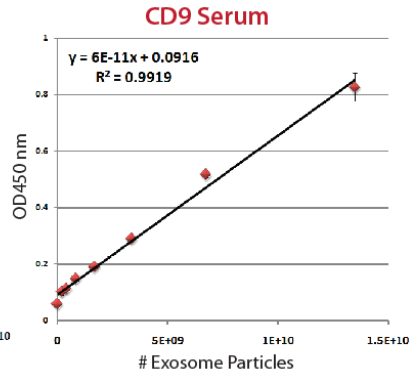
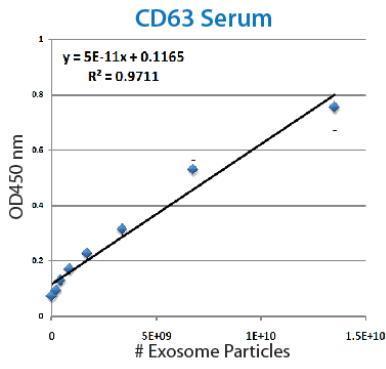


2. Dilute stock **20X Washing buffer** into **1X working Wash buffer** with purified water (each 8-well strip requires approximately 10 ml of 1X Washing solution)

ELISA assay

1. Add 50 μ l of prepared protein standards and exosome protein sample to the appropriate well of the micro-titer plate
2. Cover plate with sealing film/cover
3. Incubate the plate at 37°C from 2 hours to overnight (recommended)
4. After incubation step, invert the plate to empty all contents.
5. Wash the plate 3 times for 5 minutes each with 100 μ l **1X Wash buffer**
 - A micro-titer plate shaker is recommend for all subsequent the washing and incubation steps
 - Residual liquid should be removed by hard-tapping the plate on fresh paper towels, while taking care not to let the wells dry out completely
6. Dilute **Exosome specific primary antibody (CD63, CD9 or CD81)** 1:100 in 1X blocking buffer and add 50 μ l of to each well and incubate at room temperature for 1 hour with shaking
7. Wash the plate 3 times for 5 minutes each with 100 μ l **1X Wash buffer**
8. Dilute **Exosome validated secondary antibody** 1:5,000 1X blocking buffer and add 50 μ l to each well and incubate at room temperature for 1 hour with shaking
9. Wash the plate 3 times for 5 minutes each with 100 μ l **1X Wash buffer**
10. Add 50 μ l of **Super-sensitive TMB ELISA** substrate and incubate at room temperature for 15 to 45 minutes with shaking
 - 15 to 45 minutes substrate incubation time is optimized for the recommended exosome protein standard curve
 - Further optimization maybe required by the user for individual sample.
11. Add 50 μ l of **Stop buffer** to provide a fixed endpoint for the assay
 - Note that the initial color of a positive sample is blue and the color changes to yellow when **Stop buffer** is added
12. Quantitate results with a spectrophotometric plate reader at 450 nm absorbance

E. Sample Data



IV. Citations

As featured in: **Exosome Isolation for Proteomic Analyses and RNA Profiling** Douglas D. Taylor, Wolfgang Zacharias and Cicek Gercel-Taylor, [Serum/Plasma Proteomics, Methods in Molecular Biology, 2011, Volume 728, Part 4, 235-246, \(PDF\) »](#)

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. \(PDF\) »](#)

V. Technical References

Adachi T, Nakanishi M, Otsuka Y, Nishimura K, Hirokawa G, Goto Y, Nonogi H, Iwai N. [Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin Chem. 2010 Jul;56\(7\):1183-5.](#)

De Smaele E, Ferretti E, Gulino A. [MicroRNAs as biomarkers for CNS cancer and other disorders. Brain Res. 2010 Jun 18;1338:100-11.](#)

Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. [Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008 Jul 29;105\(30\):10513-8.](#)

Laterza OF, Lim L, Garrett-Engel PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE. [Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. Clin Chem. 2009 Nov;55\(11\):1977-83.](#)

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. [Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9\(6\):654-9.](#)

Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, de Gruijl TD, Wordinger T, Middeldorp JM. [Functional delivery of viral miRNAs via exosomes. Proc Natl Acad Sci USA; 2010 Apr 6; 107\(14\):6328-33.](#)

Mathivanan, S. and Simpson, R.J. [ExoCarta: A compendium of exosomal proteins and RNA. Proteomics. 2009.21, 4997-5000.](#)

They C, Ostrowski M, Segura E. [Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 2009. 8, 581-93.](#)

Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, Alevizos I. [Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis; 2010 Jan; 16\(1\):34-8.](#)

Luo SS, Ishibashi O, Ishikawa G, Ishikawa T, Katayama A, Mishima T, Takizawa T, Shigihara T, Goto T, Izumi A, Ohkuchi A, Matsubara S, Takeshita T, Takizawa T. [Human villous trophoblasts express and secrete placenta-specific microRNAs into maternal circulation via exosomes. Biol Reprod; 2009 Oct; 81\(4\):717-29.](#)

Taylor DD, Gercel-Taylor C. [MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol; 2008 Jul; 110\(1\):13-21.](#)

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

VI. 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:

System Biosciences (SBI)
265 North Whisman Road.
Mountain View, CA 94043

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)

Fax: (650) 968-2277

E-mail:

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

VII. Licensing and Warranty Statement

Limited Use License

Use of the ExoAB antibodies and ExoELISA Kits (*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.

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.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.

© 2011 System Biosciences (SBI), All Rights Reserved