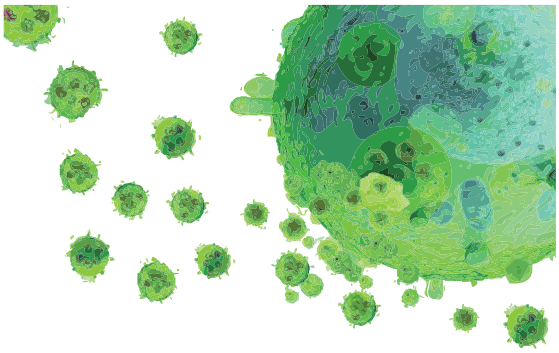


Exosome Antibodies & ELISA Kits

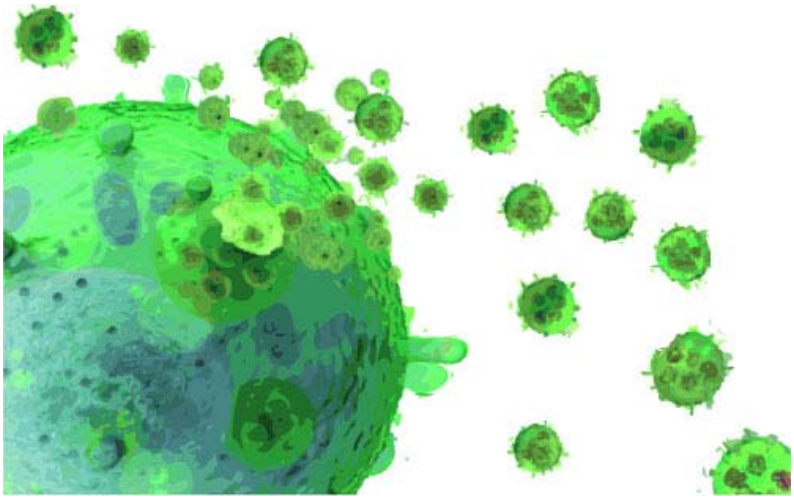
Cat #s EXOABxxx, EXOELxxx

User Manual

See page #2 for product storage conditions

(ver. 1-2011-08-16)

Contents



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

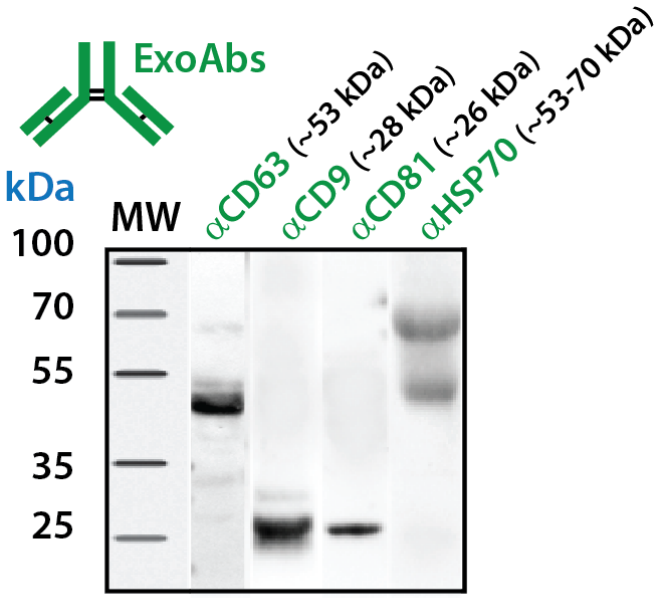
-for isolating serum exosomes for Western blotting analysis

1. If frozen, thaw 250 µl serum on ice
2. Centrifuge at 1500 × *g* for 15 minutes to remove cells and cell debris
3. Transfer sample supernatant to a centrifuge tube
4. Combine 250 µl sample + 63 µl **ExoQuick™**
5. Mix well by inversion three times
6. Place at 4ºC for at least 30 minutes
7. Centrifuge at 1500 × *g* for 5 minutes
8. Remove supernatant, keep exosome pellet
9. Centrifuge at 1500 × *g* for 5 minutes to remove all traces of fluid (take great care not to disturb the pellet)
10. Add 200 µl **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 95ºC 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



ExoQuick Exosome Serum Western Analysis

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 voulme
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 ºC 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 ºC 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

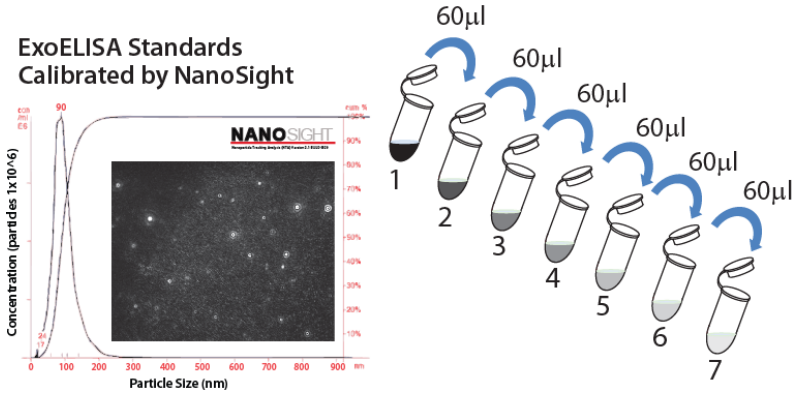
Proceed to section - **C. Exosome protein standard curve**

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:

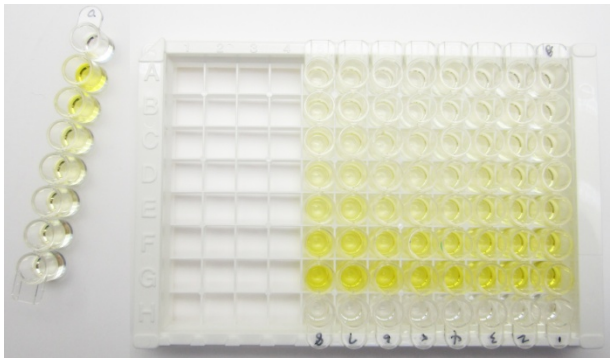
Tube	# Exosomes	Dilution factor	ExoELISA protein standard	Exosome binding buffer
0	0	Blank	-	60
1	$1.35x10^{10}$	1	60 µl	-
2	$6.75x10^{9}$	1:2	60 µl	60 µl
3	$3.37x10^{9}$	1:4	60 µl	60 µl
4	$1.68x10^{9}$	1:8	60 µl	60 µl
5	$8.44x10^{8}$	1:16	60 µl	60 µl
6	$4.21x10^{8}$	1:32	60 µl	60 µl
7	$2.10x10^{8}$	1:64	60 µl	60 µl



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.

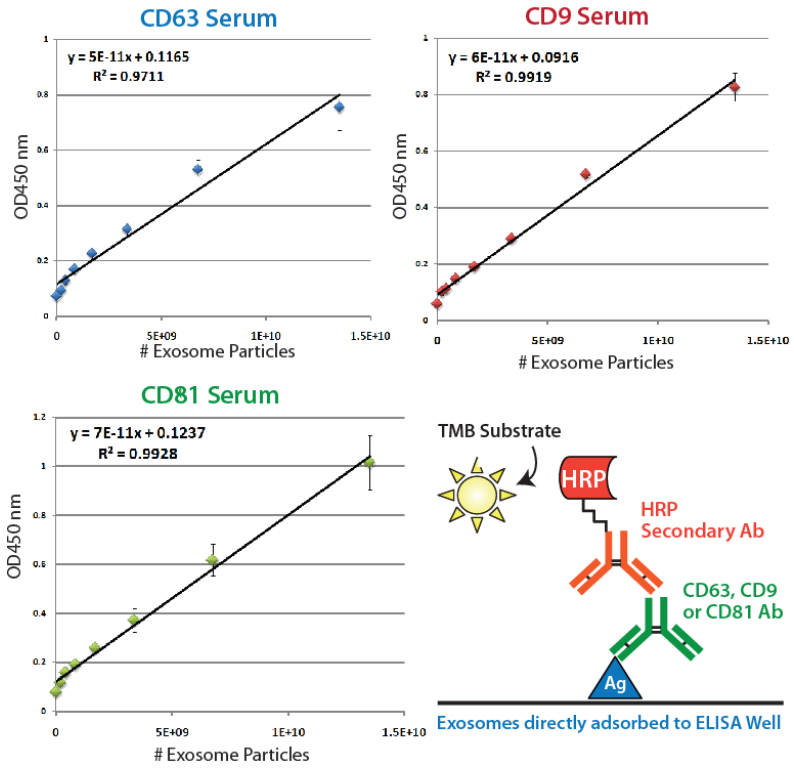


2. Dilute stock **20X Washing buffer** into **1X working Wash buffer** with purified water (each 8-well strip requires approximately 10 ml of 1X Washing solution)

ELISA assay

1. Add 50 µl 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 37ºC 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 µl **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 µl 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 µl **1X Wash buffer**
8. Dilute **Exosome validated secondary antibody** 1:5,000 1X blocking buffer and add 50 µl 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 µl **1X Wash buffer**
10. Add 50 µl 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 µl 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) »

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VI. Technical Support

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http://www.systembio.com

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

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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.

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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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