Exosome ELISA Complete Kits

EXOEL-CD9A-1, EXOEL-CD63A-1, EXOEL-CD81A-1

User Manual

See PAC for Storage Conditions for Individual Components

Version 12
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Contents

Product Description

The ExoELISA kit is designed as a direct Enzyme-Linked ImmunoSorbent Assay (ELISA). The lysed exosome particles and their proteins are directly immobilized onto the wells of the microtiter plate. After binding, the wells are coated with a blocking agent to prevent non-specific binding of the primary detection antibody. The detection (primary) antibody is added to the wells for binding to a specific antigen (e.g. CD63) protein present in the exosome lysates. A Horseradish Peroxidase enzyme linked secondary antibody (Goat anti-Rabbit) is used for signal amplification and to increase assay sensitivity. 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. The results are quantitated by a microtiter plate reader at 450 nm absorbance.

List of Components

ExoELISA kit Components	Amount
Exosome binding buffer	20 ml
20X Wash buffer	18 ml
Blocking buffer	30 ml
ExoELISA protein standard (Check the PAC for Lot-specific information)	**400 µl**
Exosome specific primary antibody (CD63) (Check the PAC for Lot-specific information)	**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

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)

Storage

The kits are shipped on blue ice and individual components should be stored according to the information below upon receipt. Properly stored kits are stable for 3 months from the date received.

Item	Storage Temperature	Notes
*Protein Standards	-20°C	**Make single use aliquots. AVOID FREEZE-THAW CYCLES!**
Other Kit Components	+4°C	

General Information

The standards included in this kit are extremely temperature sensitive, so that fluctuations or freeze-thaw cycles will negatively impact their function. Please take extreme care to make single use aliquots.

Information on how to dilute the standards is contained in the Product Analysis Certificate (PAC) and is lot-specific. Please refer to the PAC for each set of standards being used in this kit. Standards should be thawed and diluted immediately before using them.

Protocol

For exosomes precipitated with one of SBI's ExoQuick reagents

1. For exosomes precipitated with ExoQuick, resuspend the pellet in 200 µL Exosome Binding Buffer, and vortex for 15 sec.

2. Incubate at 37 ºC temperature for 20 minutes to liberate exosome proteins

3. Centrifuge at 1500 × g for 5 minutes to remove all residual precipitation solution

4. Transfer supernatant to new centrifuge tube on ice

5. Exosome protein is now ready for immobilization onto micro-titer plate

For exosomes precipitated by UltraCentrifugation

1. Carefully remove all traces of supernatant, keep exosome pellet

2. Resuspend exosome pellet in 200 µl of Exosome binding buffer and vortex 15 seconds

3. Incubate at room temperature for 10 minutes and keep on ice.

4. Exosome protein is now ready for immobilization onto micro-titer plate

Exosome protein standard curve

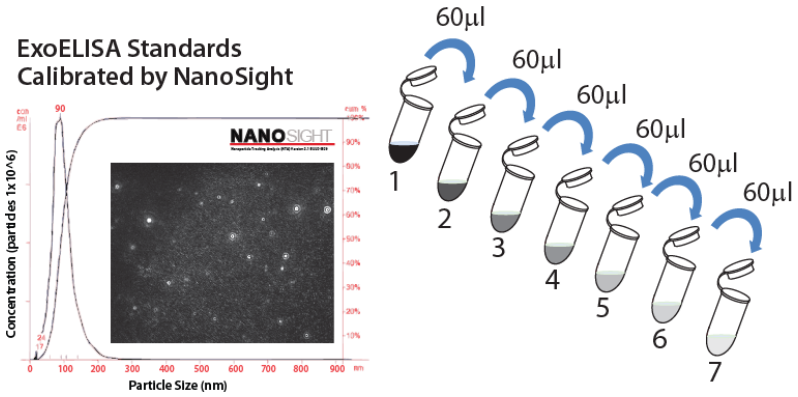
A standard curve should be prepared each time the assay is performed

1. Thaw ExoELISA protein standard on ice

2. Dilute ExoELISA protein standard by performing serial dilutions with Exosome Binding buffer in microcentrifuge tubes

3. **Below is an example of how to create a standard curve. The number of exosomes that the standard curve corresponds to is lot-specific and will be contained in the PAC that came with the kit you purchased.** Example 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



ELISA procedures

Before starting

1. The ExoELISA micro-titer plate is provided in a convenient 8 well X 12 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. Make sure to warm the Super-sensitive TMB ELISA substrate to room temperature before adding to the ELISA plate wells in step #10.

3. 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

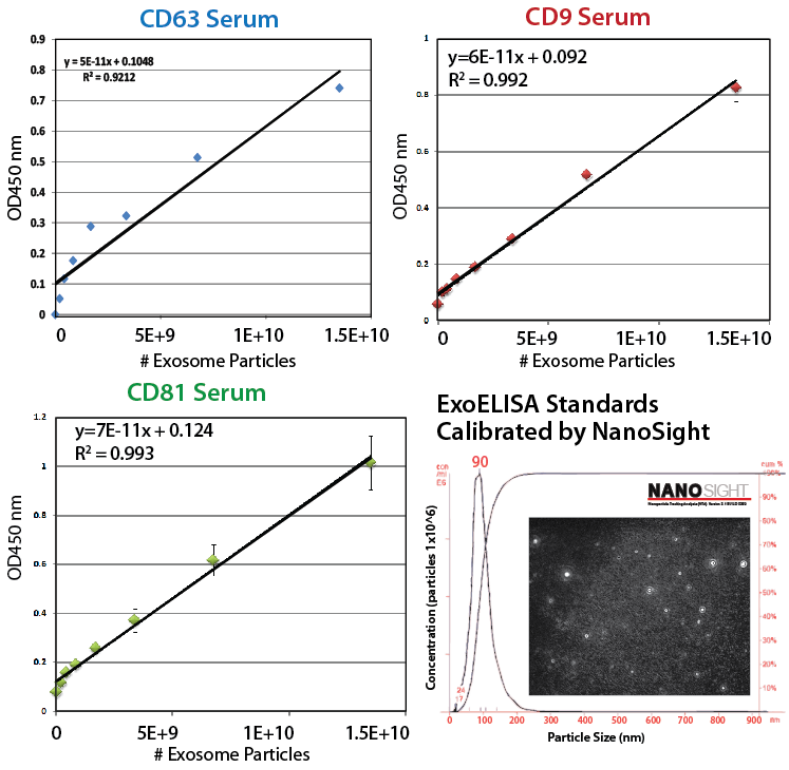
Note that the initial color of a positive sample is blue and the color changes to yellow when Stop buffer is added

12. Quantitate results with a spectrophotometric plate reader at 450 nm absorbance.

Next Steps and Related Products

Application	Related Products	Website links
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
Quantification of Exosomes		
Quantification of exosomes	EXOCET Assays	https://www.systembio.com/microrna-research/exosome-antibody/exocet-assay
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications



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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