ExoELISA-ULTRA Complete Kit (CD9 Detection)

Cat# EXEL-ULTRA-CD9-1

User Manual

See Kit Components for Individual Storage Conditions

Version 1
5/11/2022

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Product Description

The ExoELISA-ULTRA CD9 assay is a sensitive, direct Enzyme-Linked ImmunoSorbent Assay (ELISA) to quantitate exosome abundance in a given sample that can be performed within 4 hours, start to finish. Exosomes are captured intact on the high protein binding microtiter plate. The wells are incubated with an anti-CD9 primary antibody which recognizes the tetraspanin protein on the exosomal surface. A Horseradish Peroxidase enzyme linked secondary antibody is used for signal amplification. A colorimetric substrate (extra-sensitive TMB) is used for the assay read-out. The accumulation of the colored product is proportional to the amount of specific CD9 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	Storage Condition
Anti-CD9 Primary Antibody	10 µL	-20°C
HRP-conjugated Secondary Antibody	10 µL	-20°C
ExoELISA-ULTRA protein standard	10 µL	-20°C
Blocking Buffer	10 mL	4°C
Coating Buffer	20 mL	4°C
Wash Buffer (20X)	10 mL	4°C
ELISA Substrate	6 mL	4°C
Stop Buffer	6 mL	4°C
ELISA plate	1	RT

Storage

The kits are shipped at blue ice. Individual kit components are stored at different temperatures. Please review the kit component list carefully. Properly stored kits are stable for 6 months from the date received.

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)

Protocol

Exosome Precipitation

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/CG for isolation of exosomes from tissue culture media

and urine samples (EXOTC10A-1 or EXOTC50A-1) using the recommend protocols. Resuspend the pellet in sterile DPBS.

Sample Preparation

The recommended input of protein equivalent of exosomes will vary depending on the biofluid and exosome isolation method. For ExoQuick and ExoQuick-TC isolation, we recommend using 1 - 50 ug of protein input/well for the ExoELISA-ULTRA assay.

1. Use an input of 1- 50 ug protein equivalent of exosomes/well. The assay signal strength is dependent on the expression level of CD9 on the exosome membrane. We recommend the use of 5 ug of protein equivalent as a good starting point for this assay.

2. Make up the volume of exosomes (resuspended in sterile PBS) to 120 uL with the Coating Buffer (sufficient for duplicate wells).

Exosome Protein Standard Curve

A standard curve should be prepared each time the assay is performed. ***DO NOT freeze-thaw diluted standards. Make a fresh dilution of the standards (see Step 2, below) each time the assay is performed.***

1. Thaw ExoELISA-ULTRA protein standard on ice

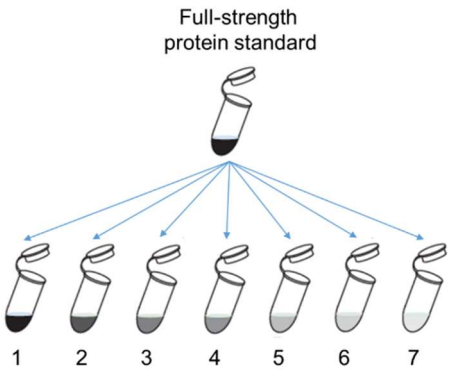
2. Prepare the "Full-strength protein standard" by adding 3 µL of the ExoELISA-ULTRA protein standard to 997 µL of Coating Buffer in a fresh microcentrifuge tube. Vortex to mix well.

3. Using the "Full-strength protein standard", prepare standard curve dilution as described in the table below in microcentrifuge tubes. Vortex to mix well.

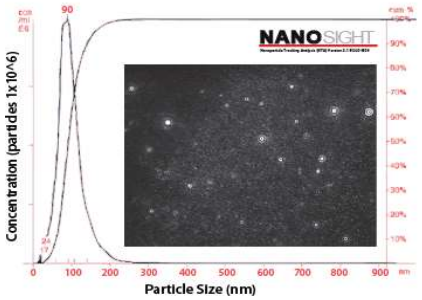
4. Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

5. ***Discard the diluted standards after use, do not freeze-thaw or reuse any of the diluted standards.***

Standard Curve Preparation



ExoELISA-ULTRA Standards calibrated by NanoSight



Tube	Exosome Abundance (particles/mL)	Full-strength protein standard	Coating buffer
1	5.42×10^{10}	120 µl	-
2	3.61×10^{10}	80 µl	40 µl
3	2.71×10^{10}	60 µl	60 µl
4	1.81×10^{10}	40 µl	80 µl
5	9.03×10^{9}	20 µl	100 µl
6	4.51×10^{9}	10 µl	110 µl
7	2.26×10^{9}	5 µl	115 µl
Blank	0	-	120 µl

ExoELISA Procedure

Before starting

1. Make sure to warm the **Super-sensitive TMB ELISA** substrate to room temperature before adding to the ELISA plate wells in step #12.
2. Dilute stock **20X Wash buffer** into **1X working Wash buffer** with purified water (each 8-well column requires approximately 10 ml of 1X Wash buffer solution).
3.

ELISA assay

1. Add 50 µl of freshly prepared protein standards (see protocol above) and exosome samples to the appropriate well of the micro-titer plate.
2. Cover plate with sealing film/cover.
3. Incubate the plate at 37ºC for 1 hours.
4. After incubation step, pipette out and dispose the content or invert the plate to empty all contents carefully ensuring there is no cross contamination between wells.
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 washing and incubation steps.

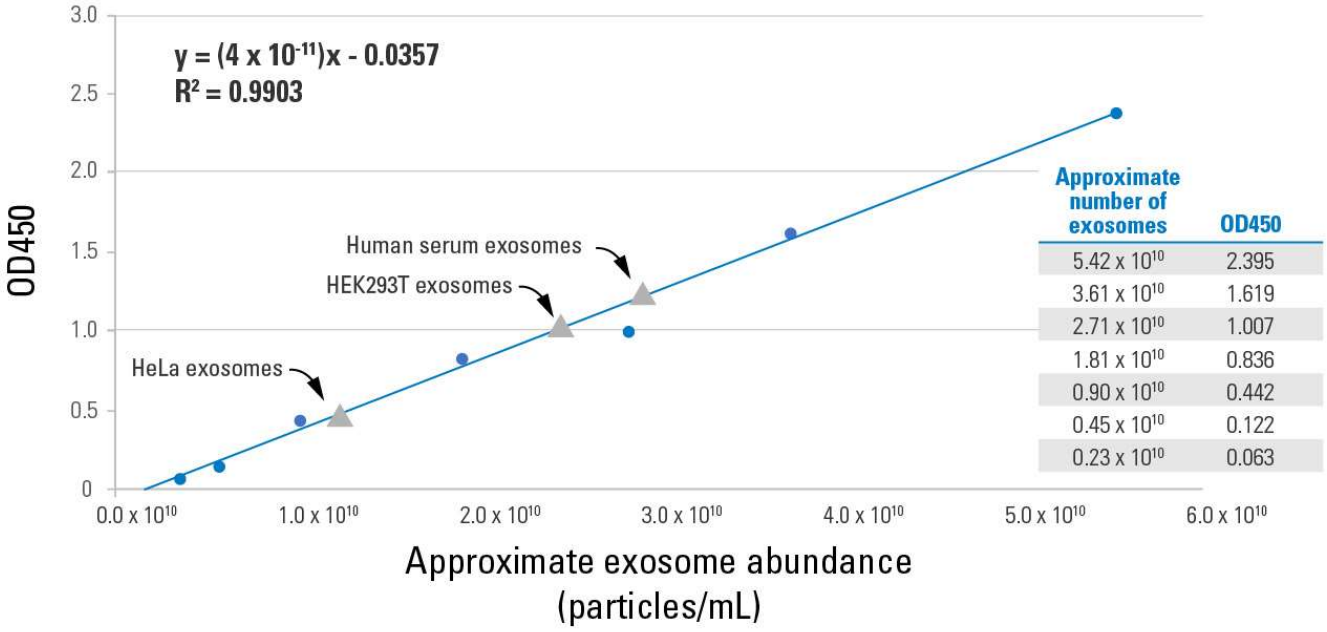
   - Residual liquid should be removed by hard-tapping the plate on fresh paper towels, while taking care not to let the wells dry out completely.

6. Dilute CD9 **primary antibody-1:750** in blocking buffer and add 50 µl to each well.
7. Incubate the plate at room temperature on shaker for 1 hour. (After incubation step, pipette out or invert the plate to empty all contents).
8. Wash the plate 3 times for 5 minutes each with 100 µl **1X Wash buffer.**
9. Dilute the **secondary antibody-1:5,000** in blocking buffer and add 50 µl to each well.

10. Incubate the plate at room temperature on shaker for 1 hour. (After incubation step, pipette out or invert the plate to empty all contents).
11. Wash the plate 3 times for 5 minutes each with 100 µl **1X Wash buffer.**
12. Add 50 µl of **Super-sensitive TMB ELISA** substrate and incubate at room temperature for 5 -15 mins with shaking*. Add 50 µl of **Stop buffer** and **read immediately** to provide a fixed endpoint for the assay. *The initial color of a positive sample is blue and the color changes to yellow when Stop Buffer is added.*
13. Quantitate results with a spectrophotometric plate reader at 450 nm.

* ***Note:*** **Optimal incubation time is dependent on lab conditions and/or instrument used. We strongly suggest running a sample set of standards to optimize the assay prior to running sensitive samples. This will help you determine the optimal conditions for your experiment.**

Example Data and Applications



Approximate number of exosomes	OD450
5.42 x 10^{10}	2.395
3.61 x 10^{10}	1.619
2.71 x 10^{10}	1.007
1.81 x 10^{10}	0.836
0.90 x 10^{10}	0.442
0.45 x 10^{10}	0.122
0.23 x 10^{10}	0.063

The ExoELISA-ULTRA CD9 standard curve shows robust linearity down to ~2 x 10^9 exosomes. OD450 nm values of commonly used samples fall well within the standard curve for the assay.

Related Products

Application	Product	Website links
Exosome/EV Isolation		
High-purity, high-yield SEC-based isolation from a range of biofluids		
High-throughput SEC-based isolation from serum and plasma, 96-well format	**SmartSEC™ HT**	https://www.systembio.com/smartsec-ht-ev-isolation-system-for-serum-plasma
Single format SEC-based isolation, validated for human serum, plasma, and CSF as well as *Aplysia californica* hemolymph	**SmartSEC™ Single**	https://www.systembio.com/smartsec-single-for-ev-isolation
Isolation from sample volumes as low as 10 µL from a range of biofulids	**SmartSEC™ Mini**	https://www.systembio.com/smartsec-mini-ev-isolation-system
High purity, polymer-based EV isolation		
Isolation from tissue culture media and other fluids	**ExoQuick-TC® ULTRA**	https://www.systembio.com/exoquick-tc-ultra-for-tissue-culture-media
Isolation from serum and plasma	**ExoQuick® ULTRA**	https://www.systembio.com/the-purest-and-highest-yielding-ev-isolation-system
General purpose, polymer-based EV isolation		
Isolation from tissue culture media and other fluids	**ExoQuick-TC®**	https://www.systembio.com/exoquick-tc
Isolation from serum and plasma	**ExoQuick®**	https://www.systembio.com/the-original-exoquick
Exosome Characterization		
Western blotting	**Exosome antibodies**	https://www.systembio.com/catalogsearch/result/?q=exoab
Antibody Arrays	**ExoCheck Arrays**	https://www.systembio.com/catalogsearch/result/?q=exoray
Exosome Quantitation		
For fast quantitation with moderate sample input requirements	**EXOCET**	https://www.systembio.com/exocet-exosome-quantitation-kit
For the most sensitive detection with very low sample input requirements	**Fluorecet**	https://www.systembio.com/fluorocet-exosome-quantitation-kit
RNA extraction from Exosomes		
Obtain high yields of total exosome/EV RNA, including small RNAs	**EVeryRNA™ EV RNA Purification System**	https://www.systembio.com/everyrna-ev-rna-purification-system
RNA extraction and profiling	**SeraMir kits**	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

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)
2438 Embarcadero Way
Palo Alto, CA 94303

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

Licensing and Warranty Statement

Limited Use License

Use of the ExoELISA-Ultra 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.

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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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System Biosciences (SBI)
2438 Embarcadero Way
Palo Alto, CA 94303

Phone: (650) 968-2200
Toll Free (888) 266-5066
Fax: (650) 968-2277

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