



ExoELISA-ULTRA Complete Kit (CD9 Detection)

Cat# EXEL-ULTRA-CD9A-1

User Manual

See Kit Components for Individual Storage Conditions

Version 1
4/24/2022

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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.

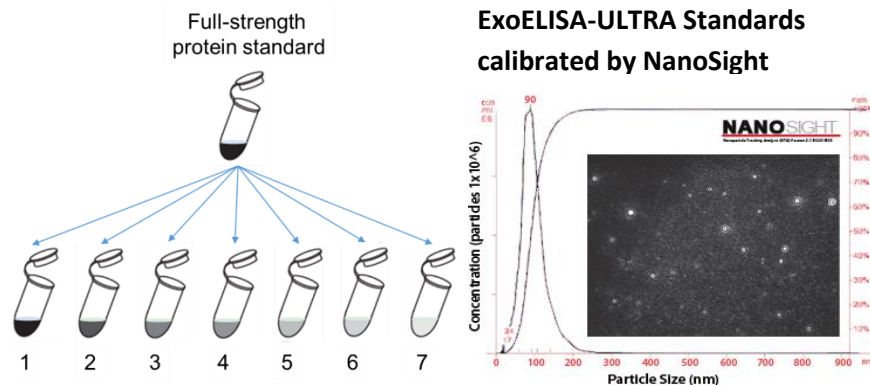
1. Use an input of 1- 50 ug protein equivalent of exosomes/well. The assay signal strength is dependent on the expression level of CD9 on the exosome membrane. We recommend the use of 5 ug of protein equivalent as a good starting point for this assay.
2. Make up the volume of exosomes (resuspended in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



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

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

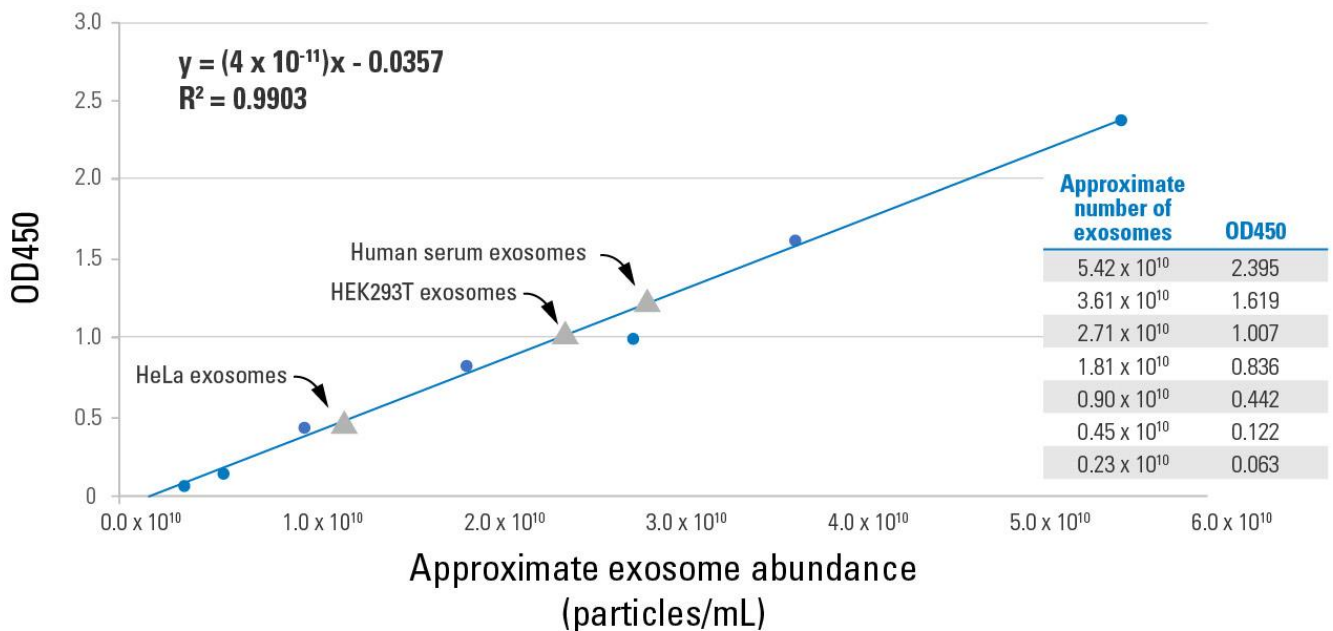
1. Add 50 μ l of freshly prepared protein standards (see protocol above) and exosome samples to the appropriate well of the micro-titer plate.
2. Cover plate with sealing film/cover.
3. Incubate the plate at 37°C for 1 hours.
4. After incubation step, pipette out and dispose the content or invert the 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 μ l **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 μ l 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 μ l **1X Wash buffer**.
9. Dilute the **secondary antibody-1:5,000** in blocking buffer and add 50 μ l 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 μ l **1X Wash buffer**.
12. Add 50 μ l of **Super-sensitive TMB ELISA** substrate and incubate at room temperature for 5 -15 mins with shaking*. Add 50 μ l 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



The ExoELISA-ULTRA CD9 standard curve shows robust linearity down to $\sim 2 \times 10^9$ 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 <i>Aplysia californica</i> 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 biofluids	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

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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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