ExoELISA-ULTRA CD81 Kit

Cat# EXEL-ULTRA-CD81-1

User Manual

See Kit Components for Individual Storage Conditions

Version 1
6/12/2018

Contents

Product Description

The ExoELISA-ULTRA CD81 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-CD81 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 CD81 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-CD81 Primary Antibody	10 uL	-20°C
HRP-conjugated Secondary Antibody	10 uL	4°C
ExoELISA-ULTRA protein standard	4 uL	-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 1-200ug of protein input/well for the ExoELISA-ULTRA assay.

1. Use an input of 1-200ug protein equivalent of exosomes/well. The assay signal strength is dependent on the expression level of CD63 on the exosome membrane. We recommend the use of 25ug of protein as a good starting point for the assay.

2. Make up the volume of exosomes (resuspended in sterile DPBS) to 120uL 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. Dilute ExoELISA-ULTRA protein standard 1:1000 in Coating Buffer in a microcentrifuge tube first (for example, add 1uL Standard to 1mL Coating Buffer). Vortex to mix well. Use this dilution as the First Standard for the standard curve.

3. Perform serial dilutions of the First Standard in Coating Buffer in microcentrifuge tubes. Vortex to mix well after each dilution.

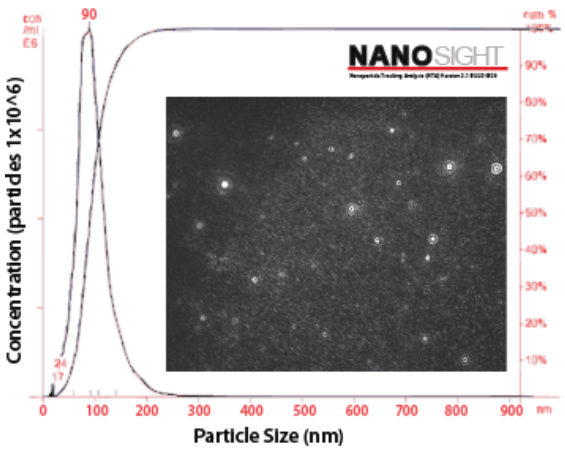
4. Suggested dilutions for making the ExoELISA-ULTRA standard curve are shown below. To run the standards in duplicate, double the recipes listed and split into two separate wells.

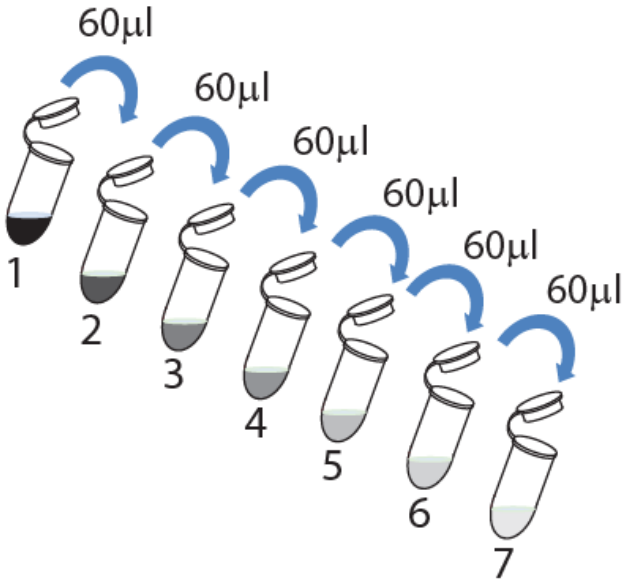
5. ***Discard the diluted standards after use, do not freeze-thaw or reuse any of the diluted standards.***

Suggested Dilutions – Standard Curve

Tube	Exosome Abundance	Dilution factor	ExoELISA-ULTRA protein standard	Coating buffer
0	0	Blank	-	60 µl
1	2.60×10^{11}	1:2	1000µl	-
2	1.30×10^{11}	1:4	60 µl	60 µl
3	6.50×10^{10}	1:8	60 µl	60 µl
4	3.25×10^{10}	1:16	60 µl	60 µl
5	1.63×10^{10}	1:32	60 µl	60 µl
6	8.13×10^{9}	1:64	60 µl	60 µl
7	4.06×10^{9}	1:128	60 µl	60 µl

ExoELISA-ULTRA Standards calibrated by NanoSight





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 #10.
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).

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 1h.
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 washing and incubation steps.

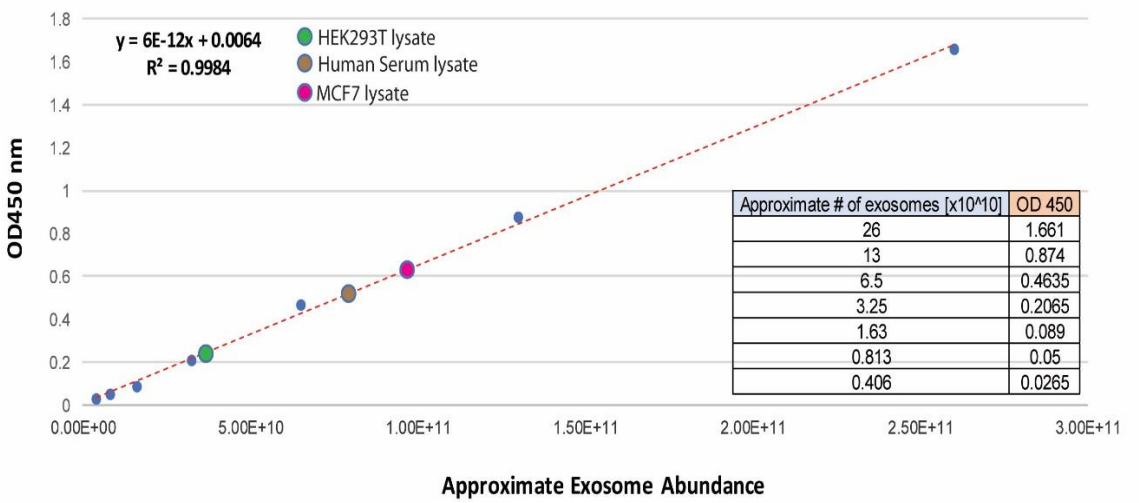
   - Residual liquid should be removed by hard-tapping the plate on fresh paper towels, while taking care not to let the wells dry out completely.

6. Dilute the **CD81 primary antibody** using the ratio 1:1000 in **Blocking Buffer** and add 50 µl of to each well.
7. Incubate the plate at room temperature for 1 hour with shaking.
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 for 1 hour with shaking.
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 10 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 # of exosomes [x10^10]	OD 450
26	1.661
13	0.874
6.5	0.4635
3.25	0.2065
1.63	0.089
0.813	0.05
0.406	0.0265

Next Steps and Related Products

Application	Related Products	Website links
Precipitation of Exosomes from other biological fluids		
Exosome Isolation from Tissue Culture Media	ExoQuickTC	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
Exosome Isolation from Plasma	ExoQuick Plasma prep and Exosome precipitation kit	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
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

Technical Support

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