ExoELISA-ULTRA Complete Kit (GroEL, For *E. coli* OMV Detection)

Cat# EXEL-ULTRA-GroEL-1

User Manual

See Kit Components for Individual Storage Conditions

Version 1
10/20/2023

Contents

Product Description

The ExoELISA-ULTRA GroEL detection assay is a sensitive, direct Enzyme-Linked Immunosorbent Assay (ELISA) to quantitate the abundance of bacterial outer membrane vesicles (OMVs) isolated from *E.coli* in a given sample. It is the first and only kit for easy quantitation and characterization of OMVs from *E. coli.* The assay can be performed within 4 hours, start to finish. OMVs are captured intact on the high protein binding microtiter plate. The wells are incubated with an anti-GroEL primary antibody which recognizes the *E.coli* GroEL, a molecular chaperonin which is among the most abundant proteins associated with bacterial OMVs. 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 GroEL antigen present in each well. The results are quantitated by a microtiter plate reader at 450 nm absorbance. To enable quantitation of OMVs carrying GroEL, the internal standard has been calibrated to OMVs isolated with different OMV isolation methods that have been analyzed by nanoparticle tracking analysis (NTA). This assay is also predicted to work with OMVs derived from *Shigella dysenteriae* and *Salmonella enterica.*

List of Components

ExoELISA kit Components	Amount	Storage Condition
Anti-GroEL Primary Antibody	8 µL	-20°C
HRP-conjugated Secondary Antibody	10 µL	-20°C
ExoELISA-ULTRA GroEL 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

OMV Isolation

For simple and quick isolation of OMVs from *E. coli* culture, we recommend using the ExoBacteria™ OMV Isolation Kit for *E.coli* and other gram-negative bacteria (Catalog# EXOBAC100A-1).

Sample Preparation

The recommended input of protein equivalent of OMVs will vary depending on the bacterial culture and OMV isolation method. For OMVs isolated with ExoBacteria™ OMV Isolation Kit, we recommend using 0.5 - 10 ug of protein input/well for the ExoELISA-ULTRA assay.

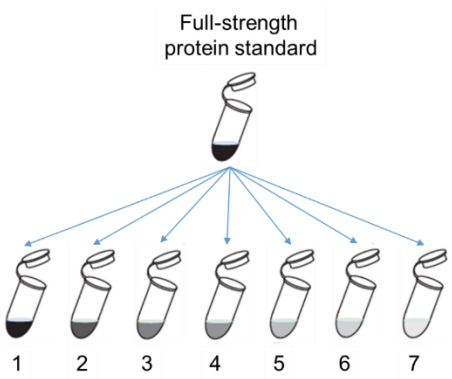
1. Use an input of 0.5 - 10 ug protein equivalent of OMVs/well. The assay signal strength is dependent on the expression level of GroEL associated with the OMVs. We recommend the use of 2 ug of protein equivalent/well as a good starting point for this assay.
2. Make up the volume of OMVs to 120 uL with the Coating Buffer (sufficient for duplicate wells).

OMV Protein Standard Curve

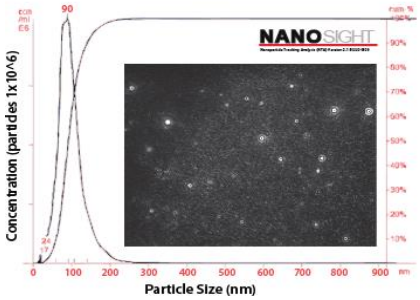
A standard curve should be prepared each time the assay is performed. ***DO NOT freeze-thaw diluted standards. Make a fresh dilution of the standards (see Step 2, below) each time the assay is performed.***

1. Thaw ExoELISA-ULTRA GroEL protein standard on ice
2. Prepare the "Full-strength protein standard" by adding 3 µL of the GroEL 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	1.96×10^{10}	120 µl	-
2	1.30×10^{10}	80 µl	40 µl
3	9.78×10^{9}	60 µl	60 µl
4	6.52×10^{9}	40 µl	80 µl
5	3.26×10^{9}	20 µl	100 µl
6	1.63×10^{9}	10 µl	110 µl
7	0.82×10^{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.

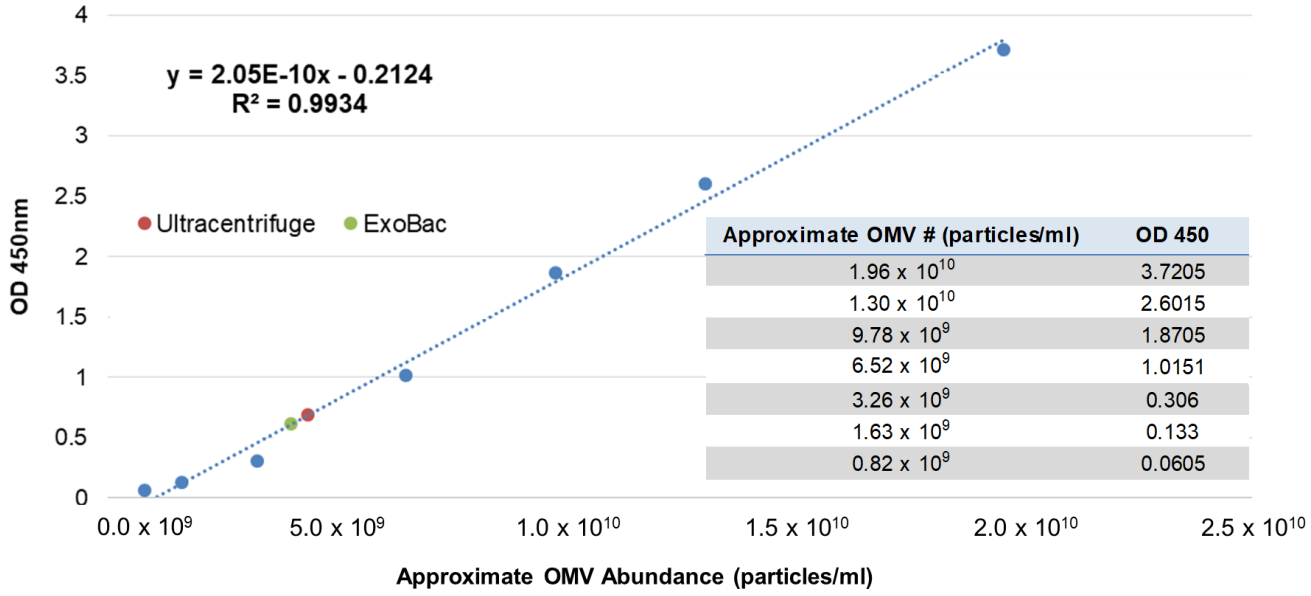
   - Residual liquid should be removed by hard-tapping the plate on fresh paper towels, while taking care not to let the wells dry out completely.

6. Dilute GroEL **primary antibody-1:1000** 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 OMV # (particles/ml)	OD 450
1.96×10^{10}	3.7205
1.30×10^{10}	2.6015
9.78×10^{9}	1.8705
6.52×10^{9}	1.0151
3.26×10^{9}	0.306
1.63×10^{9}	0.133
0.82×10^{9}	0.0605

ExoELISA-ULTRA GroEL standard curve shows robust linearity down to ~ 0.82×10^{9} OMVs. OD450nm values of OMVs isolated with common OMV isolation methods (Ultracentrifuge and ExoBacteria™ OMV Isolation Kit) fall well within the standard curve for the assay.

Related Products

Application	Product	Website links
OMV Isolation		
Clean, high-yield preps of bacterial outer membrane vesicles (OMVs)		
The only kit for easy isolation of OMVs from *E. coli* and other gram-negative bacteria	**ExoBacteria™ OMV Isolation Kit**	https://www.systembio.com/exobacteria-omv-isolation-kit-for-e-coli-and-other-gram-negative-bacteria
OMV Characterization		
EV/OMV labeling for Fluorescent NTA analysis	**ExoGlow™-NTA Fluorescent Labeling Kit (for Malvern NanoSight)**	https://www.systembio.com/exoglow-nta-fluorescent-labeling-kit-for-malvern-nanosight
	ExoGlow™-NTA Fluorescent Labeling Kit (for Particle Metrix ZetaView®)	https://www.systembio.com/exo-glow-nta-fluorescent-labeling-kit-for-particle-metrix-zetaview
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

Technical Support

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