COSMO BIO CO., LTD.

Gram-negative bacterial EV ELISA Kit

Cat. No. EVEL02

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【 I 】Background and Principle

Extracellular vesicles (EVs) are particles with lipid bilayers released from cells, which are considered to play important roles in intercellular communication[1] and are actively studied.

It has been revealed that EVs are involved not only in communication between multicellular organisms but also between microbes and between microbes and host cells.[2] EV production is an essential function for microorganisms, and elucidation of EV-mediated bacterial-bacterial and bacterial-host interactions is expected to contribute to vaccine development, studies on the effects of intestinal bacteria on hosts, drug delivery systems, and other applied fields.[3]

For quantification of EVs in multicellular organisms, ELISA kits using antibodies against marker proteins have been widely developed. In contrast, in microbes, marker proteins are not yet well identified, so quantification is mainly performed by nanoparticle tracking analysis, which requires expensive equipment.

This product captures EVs using plates coated to carry positive charges on the surface and detects them with an anti-LPS (Lipopolysaccharide) antibody as a probe.

This enables sensitive relative quantification of Gram-negative bacterial EVs without requiring expensive equipment or special techniques.

【 II 】Product Features

- Enables relative quantification of microbe-derived EVs using the same procedure as conventional ELISA
- Standards are included, allowing immediate start of experiments.
- High sensitivity enables measurement with small sample volumes.

  Limit of detection：8.0 x 10^6 particles/mL

【III】Kit Components

Storage temperature：4℃

No	Component	Volume	Quantity
1	EV Detection Plate	8-well × 12 strips	1 plate
2	E. coli EV Standard (*Escherichia coli* DH5 α strain) 5.12×10^8 particles/mL	1.0 mL *	1 vial*
3	Assay Buffer	35 mL	1 bottle
4	Wash Buffer (10X)	40 mL	1 bottle
5	Anti-LPS Antibody (100X, red cap)	120 µL	1 vial
6	HRP-labeled Secondary Antibody (200X, green cap)	150 µL	1 vial
7	Substrate Solution	12 mL	1 bottle
8	Stop Solution	6 mL	1 bottle
9	Plate Seal		3 sheets

* Standard curve can be prepared twice (n=2)

Materials required but not provided

- Micropipettes (10 – 1000 µL)
- Reservoir
- Plate reader (capable of measuring at 450 nm)
- Multichannel pipette
- Plate shaker
- Plate washer

【IV】Preparation of Reagents and Samples

【IV－1】EV Standards (prepare for 2 wells per plate

	Concentration (particles/mL)	EVs Standard	Assay Buffer	Dilution
A	5.12×10^8			1
B	2.56×10^8	250 µL of A	250 µL	2
C	1.28×10^8	250 µL of B	250 µL	2
D	6.40×10^7	250 µL of C	250 µL	2
E	3.20×10^7	250 µL of D	250 µL	2
F	1.60×10^7	250 µL of E	250 µL	2
G	8.00×10^6	250 µL of F	250 µL	2

Preparation of Standard Solutions

1. Add 250 µL of Assay Buffer to 250 µL of the EVs Standard Solution included in the kit (A in the table above) to make a 2-fold dilution. Mix well to obtain solution B.

2. Prepare subsequent 2-fold serial dilutions in the same manner.

3. Use 100 µL of each dilution per well.

Note: Since each concentration is measured in duplicate (n = 2), 200 µL of each dilution will be required.

Caution: Prepare the EVs Standard Solution freshly at the time of use, and only in the amount needed.

【IV】Reagents and Sample Preparation (cont.)

【IV－2】Wash Buffer

・Dilute Wash Buffer (10X) 10-fold with purified water.

Example: For one plate, dilute 40 mL Wash Buffer (10X) with 360 mL purified water.

【IV－3】Anti-LPS Antibody

・Dilute Anti-LPS Antibody (100X) 100-fold with Assay Buffer.

Example: For one plate, dilute 100 µL Anti-LPS Antibody (100X) with 10 mL Assay Buffer.

Caution: Prepare the antibody solution freshly at the time of use, and only in the required amount.

【IV－4】HRP-labeled Secondary Antibody

・Dilute HRP-labeled Secondary Antibody (200X) 200-fold with Assay Buffer.

Example: For one plate, dilute 50 µL HRP-labeled Secondary Antibody (200X) with 10 mL Assay Buffer.

【IV－5】Samples

・Dilute samples at least 2-fold with Assay Buffer so that the total reaction volume becomes 100 µL.

・EVs may not be captured if the solvent contains high salt concentrations. Replace or dilute the solvent with phosphate buffer or an equivalent buffer to reduce the salt concentration to 150 mM or lower.

・For some samples, the relationship between concentration and absorbance may not be linear even if within the calibration curve. Prepare multiple dilutions to confirm linearity.

【V】Assay Procedure

① Bring EV Detection Plate and reagents to room temperature.

② Prepare EV standards as described in [IV-1].

③ Add 100 µL of prepared EV standards (8.00×10^6 to 5.12×10^8 particles/mL) or sample solution to each well.

④ Seal the plate and shake with a plate shaker (800 rpm, 30 seconds).

⑤ Incubate at room temperature for 1.5 hours or overnight at 4° C.

⑥ Remove reaction solution completely, add 300 µL Wash Buffer (see [IV-2]) to each well, and wash. Repeat 3 times.

⑦ Add 100 µL of diluted Anti-LPS Antibody (see [IV-3]) to each well.

⑧ Seal the plate, shake at 800 rpm for 30 seconds, and incubate at room temperature for 1.5 hours.

⑨ Remove antibody solution completely, wash each well 3 times with 300 µL Wash Buffer.

⑩ Add 100 µL of diluted HRP-labeled Secondary Antibody (see [IV-4]) to each well.

【V】Assay Procedure (cont.)

⑪ Seal the plate, shake at 800 rpm for 30 seconds, and incubate at room temperature for 1.5 hours.

⑫ Remove antibody solution completely, wash each well 3 times with 300 µL Wash Buffer.

⑬ Add 100 µL of Substrate Solution to each well and incubate at room temperature for 10-15 minutes, or until sufficient color develops.

⑭ After confirming the color intensity, add 50 µL of Stop Solution to each well.

⑮ Measure absorbance of each well with a plate reader at 450 nm.

⑯ Draw a calibration curve using the concentrations and absorbance of standards, and calculate the concentrations of the samples.

【VI】References

1. Y. Naito, Y. Yoshioka, Y. Yamamoto and T. Ochiya: Cell Mol Life Sci., 74, 697 (2017).
2. Obana, N., Kurosawa, M., Toyofuku, M. & Nobuhiko, N. Biogenesis and Functions of Membrane Vesicles Actively Produced by Microbes. KAGAKU TO SEIBUTSU 54, 812–819 (2016).191.
3. Obana, N. & Nomura, N. Functions and biosynthesis of membrane vesicles produced actively by Gram-positive bacteria. Japanese J. Lact. Acid Bact. 27, 10–16 (2016).

【VII】Notes

Examples are available online.

Please search "Gram-negative bacterial EV ELISA" on our website.

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