CD9 Exosome ELASA Kit, Human

For Research Use Only Cat. No.: APCD9

Background

Exosomes, small membrane-bound vesicles released by cells, are vital mediators of intercellular communication, transporting diverse molecular cargo such as proteins and microRNAs. These vesicles, typically ranging from 30 nm to 200 nm in size, are abundant in bodily fluids like saliva and blood, as well as in cell culture mediums. They are encapsulated by a lipid bilayer membrane and feature surface proteins, including members of the tetraspanin family like CD9 and CD63, which serve as markers for their identification.

Traditional methods for quantifying exosomes often rely on antibody-based techniques such as the Antibody ELISA. However, these methods can be costly and may lack the desired specificity, leading to potential cross-reactivity issues.

In contrast, a more targeted approach involves the use of aptamers in assays like the Aptamer ELISA. Aptamers, single-stranded DNA or RNA molecules, exhibit high affinity and specificity for their target molecules. In the context of exosome detection, aptamers can serve as alternative recognition elements to antibodies.

The Aptamer ELISA(ELASA) for exosome quantification utilizes high-performance aptamers specifically targeting exosome surface markers like CD9 or CD63. This innovative approach provides a direct and efficient method for quantifying exosomes in body fluids or cell culture supernatants after purification steps. By harnessing the unique binding properties of aptamers, this assay offers a cost-effective and specific solution for exosome research and diagnostics.

Product Description

In this Indirect ELASA assay, exosomes are captured intact on the high protein binding microtiter plate. Then, CD9 probe is added, which binds to CD9 on the captured exosomes. Following wash steps, HRP-conjugated Secondary Probe and TMB solution is added, leading to a color change proportional to the amount of exosomes. The colorimetric signal is quantified using a microplate reader, providing a sensitive and specific method for exosome quantification.

Kit Components

	Component	Amount
1	Exosome Protein Standard (Blue cap)	30 μL
2	Coating Buffer	20 mL
3	Blocking Buffer	40 mL
4	CD9 Probe (Green cap)	10 μL
5	HRP-conjugated Secondary Probe (Red cap)	10 μL
6	Probe Dilution Buffer	40 mL
7	Wash Buffer (10X)	50 mL
8	Substrate Buffer	12 mL
9	Stop Buffer	12 mL
10	96 Well Plate	1 plate (16-well x 6 strips)

*** Storage temperature : 4 ℃**

*** Sufficient to create 3 standard curves with n = 2.**

*** Exosome Protein Standard Period After Opening：3 months.**

*** If the 96 Well Plate is not used completely in one experiment, remove unused strips and remain in the aluminum foil bag.**

*** Store reagents immediately after use.**

Materials Not Included in the Kit

1. Microtiter plate sealing film/cover
2. Micropipettes (10 to 1000 μL)
3. Multichannel micropipette (recommended)
4. Multichannel micropipette reservoir
5. Microtiter plate shaker
6. Microplate reader (capable of measuring at a wavelength of 450nm)
7. Plate washer

Preparation of Reagents and Samples

A. Standard Preparation

Create a new standard curve each time. Avoid freezing-thawing standards. Should not be vortexed to avoid degradation. Make fresh dilutions of the standards for each assay.

A-1 Exosome Standard Stock Preparation

1. Thaw Exosome Protein Standard on ice.
2. Prepare the "**Exosome Standard Stock**" in a fresh microcentrifuge tube, and then mix well.

	Exosome Abundance (particles/mL)	**Exosome Standard**	**Coating buffer**
S1	1×10^{11}	5 µL of Exosome Protein Standard (6×10^{11} particle/mL)	25 µL
S2	1×10^{10}	10 µL of S1	90 µL
S3	1×10^{9}	10 µL of S2	90 µL
Exosome Standard Stock	8×10^{7}	80 µL of S3	920 µL

A-2 Standard Dilutions Preparation

1. Using the "**Exosome Standard Stock**", prepare standard curve dilution as described in the table below in microcentrifuge tubes, and then mix well.
2. Use 100 µL for measurement, using 2 wells for each solution (n=2).

Tube	**Exosome Abundance (particles/mL)**	**Exosome Standard Stock**	**Coating buffer**
1	8×10^{7}	220 µL	-
2	6.4×10^{7}	240 µL	60 µL
3	5.12×10^{7}	192 µL	108 µL
4	2.56×10^{7}	96 µL	204 µL
5	1.28×10^{7}	48 µL	252 µL
6	6.4×10^{6}	24 µL	276 µL
7	2.5×10^{6}	9.4 µL	290.6 µL
Blank	0	-	250 µL

*** Discard the Standard Dilutions after use, do not freeze-thaw or reuse.**

B. Sample Preparation

The recommended input of protein equivalent of exosomes will vary depending on the exosome production method and isolation method. For TFF (Tangential Flow Filtration) isolation, we recommend using **0.02 - 0.6 µg of protein input/well** for the assay.

1. Dilute the sample to **0.02 - 0.6 µg with sterile PBS in 10 µL**.
2. Add **90 µL with the Coating Buffer**.

***** Each sample should be assayed in duplicate.

C. Wash Buffer Preparation

・Dilute **Wash Buffer (10X)** into **1X working Wash buffer** with ddH2O.

e.g. For 1 plate, add 405 mL of ddH2O to 45 mL of Wash Buffer (10X) and mix well.

Trouble Shooting

Problem	Possible Cause	Solution
Weak or no signal	The protein amount is added into well insufficiently	Ensure extract contains enough amount of proteins
	Incubation time and temperature is incorrect	Ensure the incubation time and temperature described in the protocol are correctly followed
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
High background	Contaminated wash buffer	Make fresh wash buffer
	Insufficient washing	Ensure the number of washes described in the protocol are correctly followed
Anomalous signal	Reagents not fresh or not at the correct pH	Use freshly prepared solutions and reagents
	Reagent added in incorrect order, or incorrectly prepared	Review protocol and check the concentration of reagents
	Bubbles in the wells	Make sure you remove air bubbles within wells
	Uneven temperature around work surface	Avoid incubating plate in areas where environmental conditions vary
	Filth at the bottom of 96 well plate	Make sure the surface is clean before adding the reagents into wells
	The well or plate abnormality in the experimental process	Ensure to dry the wells thoroughly before adding the substrate. Do not let the wells dry out during incubation. Remove any residual liquid and fingerprints from the bottom of the plate to avoid affecting OD values.
Low OD values	Incorrect microplate reader settings	Check wavelength and filter settings, preheat reader. Check wavelength and filter settings, preheat reader.
Low sensitivity	Improper storage of the ELISA kit	Store reagents as per instructions
	No stop solution added before reading	Add stop solution before reading OD

Tips

- ✓ Pipet the reagents to the sides of the wells to avoid possible contamination.
- ✓ Use fresh pipet tips for each sample, standard, and reagent.

Procedure

A. Before starting

1. Fresh dilute CD9 Probe (2500X) to 2500-folds in Probe Dilution Buffer before step # 6.

e.g. For 1 plate, add 4 µL of CD9 Probe (2500X) into 10 mL of Assay Buffer. Mix by inverting the tube.

2. Fresh dilute HRP-conjugated Secondary Probe (2500X) to 2500-folds in Probe Dilution Buffer before step # 8.

e.g. For 1 plate, add 4 µL of HRP-conjugated Secondary Probe (2500X) into 10 mL of Assay Buffer. Mix by inverting the tube.

B. ELASA assay

1. Add 100 µl of freshly prepared **Standard Dilutions (see protocol above)** and exosome samples to the appropriate well of the **96 Well Plate**.
2. Seal the plate with plate seal and incubate it at 4℃ overnight.
3. Remove the plate seal, discard all the reaction solution. Wash each well with 350 µL of **1X Wash buffer**, and then discard the buffer carefully. Repeat this step for 3 times. After the final wash, empty the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

* Note: **Do not** use **suction/aspirate** to directly suck the bottom of the well.

4. Add 300 µL of **Blocking Buffer** to each well. Seal the plate with plate seal and incubate it at room temperature for 1 h on the Microtiter plate shaker (100 rpm).
5. Remove the plate seal, discard all the reaction solution. Wash each well with 350 µL of **1X Wash buffer**, and then discard the buffer carefully. Repeat this step for 3 times. After the final wash, empty the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

* Note: **Do not** use **suction/aspirate** to directly suck the bottom of the well.

6. Add 100 µL of dilute **CD9 Probe (1X)** to each well. Seal the plate with plate seal and incubate it at room temperature for 1 h on the Microtiter plate shaker (100 rpm).
7. Remove the plate seal, discard all the reaction solution. Wash each well with 350 µL of **1X Wash buffer**, and then discard the buffer carefully. Repeat this step for 3 times. After the final wash, empty the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

* Note: **Do not** use **suction/aspirate** to directly suck the bottom of the well.

8. Add 100 µL of dilute **HRP-conjugated Secondary Probe (1X)** to each well. Seal the plate with plate seal and incubate it at room temperature for 1 h on the Microtiter plate shaker (100 rpm).
9. Warm the **Substrate Buffer** to room temperature.
10. Remove the plate seal, discard all the reaction solution. Wash each well with 350 µL of **1X Wash buffer**, and then discard the buffer carefully. Repeat this step for 3 times. After the final wash, empty the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash

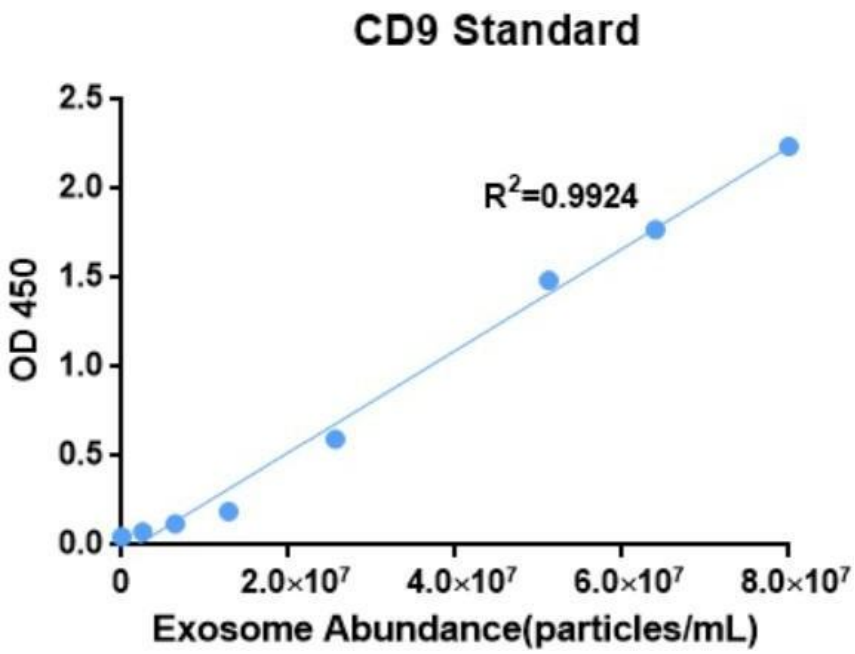
buffer.

* Note: **Do not** use **suction/aspirate** to directly suck the bottom of the well.

11. Add 100 μL of **Substrate Buffer** to each well, and then incubate the plate in dark at room temperature for 15 minutes. Add 100 μL of **Stop Buffer** to each well and read immediately.

* Note: The initial color of a positive sample is blue and the color changes to yellow when Stop Buffer is added.

12. Measure the absorbance with a microplate reader at 450 nm.
13. Plot the standard solutions data as mean OD 450.
14. Calculate each sample concentration.

* Note: The ideal incubation time varies depending on laboratory conditions and/or the instrument utilized. It's highly recommended to run a sample set of standards beforehand to fine-tune the assay before analyzing sensitive samples. This preliminary step assists in identifying the optimal experimental conditions for your study.

Example of Standard curve