ExoELISA-Step

Enzyme Immunoassay for the detection and quantification of Exosomes.

REFERENCE	DESCRIPTION	SIZE
Exo2506	ELISA assay for detection and quantification of exosomes from cell culture	96 test
Exo2508	ELISA assay for detection and quantification of exosomes from human serum	96 test

1. INTRODUCTION

Exosomes are small extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB)[1], with the plasma membrane. They are thought to provide a means of intercellular communication[2,3] and of transmission of macromolecules between cells allowing the spread of proteins, lipids, mRNA, miRNA and DNA and as contributing factors in the development of several diseases. Exosomes can also modulate cancer microenvironment[4] and the immune response [5,6].

2. PRODUCT DESCRIPTION

ExoELISA-Step is an assay based on the use of antigens or antibodies labeled with an enzyme, so that the resulting conjugates have both immunological and enzymatic activity. As one of the components (antigen or antibody) is labeled with an enzyme and insolubilized on a support (immunosorbent) the antigen-antibody reaction will remain immobilized and therefore, is easily revealed by the addition of a specific substrate. When acting on the enzyme a simple or quantifiable view will produce an observable color using a spectrophotometer or a colorimeter. The following protocol describes how an indirect ELISA is performed.

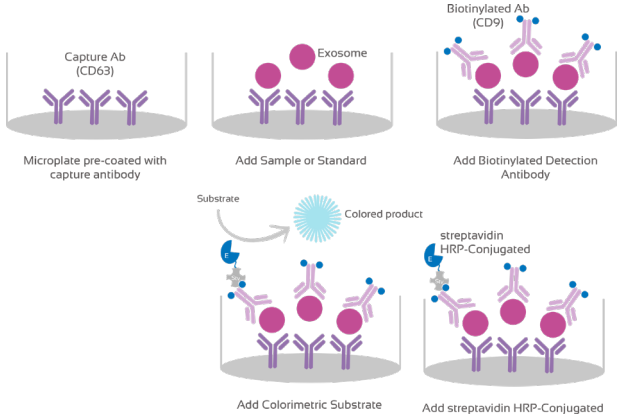

Figure 1: Direct ELISA graphical representation.

3. REAGENTS PROVIDED

DESCRIPTION	COMPONENTS	AMOUNT
Immunoplate	Immunoplate 96-well format	1 plate
EXOSTEP Standard for assay calibration	Lyophilized Exosome Standards in according with kit. Ref: Exo2506 Contains PC3 Lyophilized Exosome Standard. Ref Exo2508 contains Serum/Plasma Lyophilized exosome standard.	1 vial (100µg)
Washing buffer 20x	HBS 0.05% Tween washing solution.	50ml
Buffer diluent IX	Antibody and sample dilution buffer (IX- Ready to use). Buffer that minimizes non-specific cross-reactivity and matrix interference, with blue dye. Contains CMIT/MIT 3:1 as a preservative.	25ml
Primary Antibody (100X)	Monoclonal Anti-Human CD9 biotin conjugated	120ul
HRP-Conjugated (100X)	HRP-conjugated antibody	120ul
Substrate solution	TMB	1 bottle (12ml)
Stop solution	H2SO4 0,5M	1 bottle (12ml)
Sealing film	Protective film	2 units protective film

4. APPROPIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2°C and 8°C. The kit is stable until the expiry date stated on the vial label if kept at 2-8°C. Do not use after the date indicated.

DESCRIPTION	PREPARATION	STATE	STORE
Immunoplate	Immunoplate 96-well format		2/8°C
Standard for assay calibration	Lyophilized Exosome Standards in according with kit	Lyophilized	2/8°C
Washing buffer 25x	HBS 0.05% Tween washing solution.	Liquid	2/8°C
Buffer diluent IX	Antibody and sample diluent	Liquid	2/8°C
Primary Antibody	Monoclonal Anti-Human CD9 biotin conjugated	Liquid	2/8°C
HRP-Conjugated	HRP-conjugated antibody	Liquid	2/8°C
Substrate solution	TMB	Liquid	2/8°C
Stop solution	H2SO4 0,5M		

5. REAGENTS NO PROVIDED

- Calibrated spectrophotometer for Reading ELISA plates at 450 nm and 620 nm.
- Adjustable, calibrated micropipettes covering a range of 1-100 µL and corresponding disposable pipette tips.
- Automatic plate washer: recommended. Plate washing can also be performed manually.
- Incubator: for incubation of the microplate at +37°C.
- Distilled or deionized water.
- Timer.
- Disposable gloves.
- Waste container for biological substances.

6. EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

7. BIOSAFETY LEVEL 1

Biosafety classification is based on 2000/54/EC Directive from the European Council. Customer has to ensure that their facilities comply with biosafety regulations for their own country.

8. RECOMMENDATIONS AND WARNINGS

a. Reagents should be protected from prolonged exposure to light throughout this procedure.

b. The samples should be treated with appropriate handling procedures.

c. Do not use after the expiry date indicated on the vial.

d. Deviations from the recommended procedure could invalidate the analysis results
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e. For professional use only.

9. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

10. STANDARD PREPARATION

1. Resuspend the standard vial in 100ul of deionized water. Let it reconstitute for at least 15 minutes. Each lyophilized vial contains 100ug of isolated exosomes. It is recommended that it firs well contains 15 ug.

2. Although it is recommended the first well contains 15 ug, each laboratory will have to determine its own dilutional range to obtain the regression model, which will preferably be linear.

3. Perform 1:2 serial dilutions. It is recommended to use two replicates for each dilution and between 7 and 11 concentration points (figure 2).

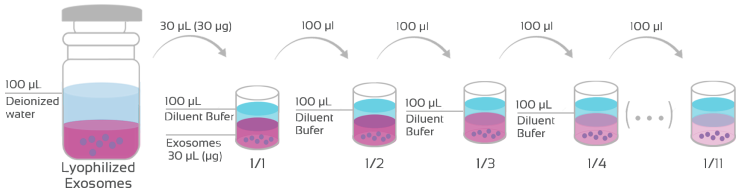

Figure 2: Graphical Representation of the serial dilution of the Standard included in the kit.

In the initial determination of the dilutional range, more points can be used, and the range can be extended to make it 20-30% wider with the intention of eliminating some of the points and obtaining the widest possible linear range (figure 3).

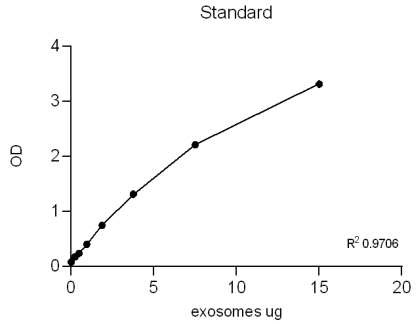

Figure 3: Example of linear range with the exosome standard.

11. SAMPLE PREPARATION

1. Purified exosomes: dilute your sample in HBS.

2. Serum: Centrifuge the samples at 500xg 10', 2000xg 10' and 14000xg 30'. Dilute the samples to 1/2 in HBS.

3. Cell culture supernatant: Centrifuge the samples at 500xg 10', 2000xg 10' and 14000xg 30'. Dilute your sample in HBS.

12. PROTOCOL

1	Sample adittion	Add 100 µl of the prepared sample (1: 100 dilution with sample diluent) into the individual wells of the microplate. It is recommended to use two wells per sample. Incubate for 2 hours at +37° C (or overnight at + 4°C) . When the process is manual, cover the microplate with one of the protective sheets provided.
2	Washing	If necessary, remove the protective foil. Empty the wells and then wash 4 times using 300 µl of 1X wash buffer for each wash. Leave wash buffer in each well for 30-60 seconds for each wash cycle. After washing, completely remove all liquid from the microplate by tapping it on absorbent paper with the openings facing down to remove all residual wash buffer.
3	Antibodies binding	Add 100ul/well of the primary antibody dilution (1:100 in sample diluent). Seal the plate with parafilm and incubate an hour at +37°C.
4	Washing	If necessary, remove the protective foil. Empty the wells and wash as described previously (step 2).
5	Streptavidin adittion	Add 100ul/well of the SA-HRP antibody dilution (1:100 in sample diluent). Seal the plate with parafilm and incubate 30 minutes at +37°C.
6	Washing	If necessary, remove the protective foil. Empty the wells and wash as described previously (step 2).
7	Substrate incubation	Add 100 µl of the Chromogen Substrate Solution (TMB) to each well of the microplate. Incubate for 10 minutes at room temperature (+ 18 ° C and + 24 ° C) and protected from light.
8	Stopping	Add 100 µl of the stop solution (1X - ready to use) to each well, trying to follow the same order in which the substrate solution was added.
9	Absorbance measurement	Measure the optical densities (O.D.) of each well on a microplate spectrophotometer at 450 nm, within 30 minutes of adding the Stop Solution. Before measurement, carefully shake the plate to ensure a homogeneous distribution of the solution.

13. REFERENCES

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