ExoELISA-Step

Enzyme Immunoassay for the detection and quantification of Exosomes.

REF	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 (capture antibody) is coating a support (immunosorbent) the antigen-antibody reaction will remain immobilized and therefore, is easily revealed by the addition of a specific substrate.

After incubation with the sample and the subsequent washing step, an antigen-specific biotin-antibody (detector or primary antibody) is added, which after an intermediate wash to remove possible excess antibody is revealed with the aid of an HRP-conjugated antibody (HRP-conjugated or secondary antibody). Upon addition of the substrate solution (TMB), a colorimetric reaction mediated by the HRP enzyme is observed with the naked eye. This reaction can be quantified using a spectrophotometer or colorimeter.

The following protocol describes how an indirect ELISA is performed.

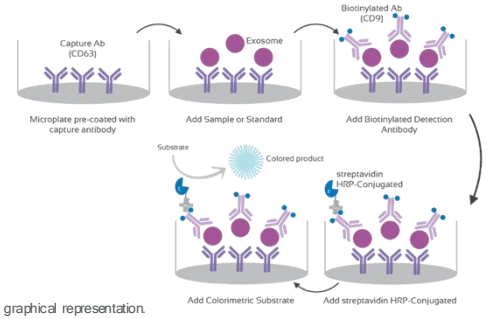

Figure 1: indirect ELISA graphical representation.

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 1X	Antibody and sample dilution buffer (1X- Ready to use). Buffer that minimizes non-specific cross-reactivity and matrix interference, with blue dye. Contains CMIT/MIT 3:1 as a preservative.	50ml
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	H_2SO_4 0,5M	1 bottle (12ml)
Sealing film	Protective film	2 units protective film

Revision nr. 7 | Emission date: 21/12/2021

4. APPROPIATE STORAGE AND HANDLING CONDITIONS

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

DESCRIPTION	PREPARATION	STATE	STORE
Immunoplate	Immunoplate 96-well format	Dried	2/8°C
Standard for assay calibration	Lyophilized exosome standards in according with kit	Lyophilized	2/8°C
Washing buffer 20x	HBS 0.05% Tween washing solution	Liquid	2/8°C
Buffer diluent 1X	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	H_2SO_4 0,5M		

5. REAGENTS NOT 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

- Reagents should be protected from prolonged exposure to light throughout this procedure.
- The samples should be treated with appropriate handling procedures.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- For professional use only.
- Pay attention if the kit is used in combination with annexin assays. The buffer used to work with annexin generates non-specificity with the ExoStep kit.

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, APPROPIATE STORAGE AND HANDLING CONDITIONS

The kit contains a vial of lyophilized exosomes (100 µg) with which you can build your calibration curve.

Lyophilized exosomes can be stored between 2ºC and 8ºC for up to 2 years without functional compromise. Immunostep recommends storing small, single -use aliquots of reconstituted exosomes, at - 20ºC for up to one month or at - 80ºC for longer periods, preferably in locations in frost-free freezers, without appreciable temperature fluctuation. This will minimize protein denaturation that can occur after multiple freeze/thaw cycles.

Reconstituted exosomes, store properly, are functionally guaranteed for up to six months from date of reconstitution. Any unfrozen and/or unused exosome standard can be stored at 4ºC for short term use (<1 week), and should not be re-frozen.

To carry out the dilutions for the construction of the standard curve follow the protocol below:

1. Resuspend the standard vial in 100 µl of deionized water and let it reconstitute for at least 15 minutes. It is recommended to do each of the dilutions in duplicate to minimize the error. We will make the dilutions in Eppendorf and then, we will transfer them to the ELISA plate wells (100 µl/well).
2. To perform the dilution in duplicate, we will add 60 µl of the lyophilized resuspended exosomes in Eppendorf nº1. We will complete with 340 µl of dilution buffer and homogenize the sample. The rest of Eppendorf must contain 200 µl of dilution buffer.
3. We need to mix 200 µl from Eppendorf nº1 (1/1 dilution) to Eppendorf nº2 (1/2 dilution).

   Repeat this process until the 1/1024 dilution is completed (figure 2). Last well should contain only diluent buffer. This is the point that we will use as blank.

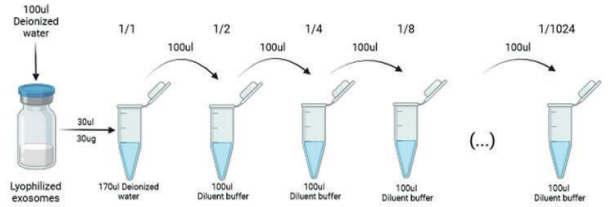

Figure 2: Graphical Representation of the serial dilution of the Standard included in the kit.

We recommend between 7 and 11 points to have a better model fit.

The curve should always have a blank value, in order to avoid future problems with sample interpolation.

well	ug/ml
1	150
2	75
3	37,5
4	18,8
5	9,4
6	4,7
7	2,3
8	0

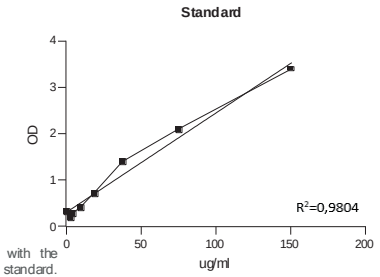

Figure 3: Example of linear range with the exosome standard.

11. SAMPLE PREPARATION

Depending on the reference to be used, we recommend processing the sample as follows:

A. Sample pretreatment for direct exosome detection on cell culture supernatant.

The sample pretreatment for direct exosome detection from cell culture supernatant is not recommended for detection of exosomes from any other body fluids.

To ensure that detected exosomes originate from your cells of interest, culture the cells with exosome depleted fetal bovine serum (FBS), because normal FBS contains extremely high levels of exosomes that will contaminate the cells derived exosomes.

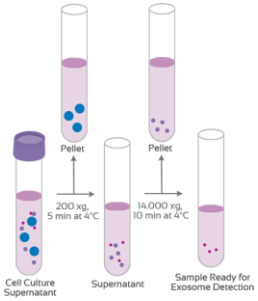

Figure 4: Cell Culture Supernatant pretreatment workflow for direct exosome detection. Please, refer to https://immunostep.com/exosomes/ for additional technical information.

B. Sample pretreatment for direct exosome detection on Serum/Plasma.

The sample pretreatment for direct exosome detection from plasma or serum is not recommended for detection of exosomes from any other body fluids or cell culture media.

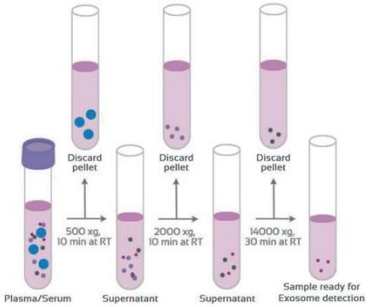

Figure 5: Serum Supernatant pretreatment workflow for direct exosome detection.

If the OD of the sample is greater than the highest concentration point, we recommend to perform a dilution until the values fit the model we are following.

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 + 4ºC) . 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 +37ºC.
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 +37ºC.
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. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

14. EXPLANATION OF SYMBOLS

	Fluorochrome
REF	Product reference
Σ	Content for <n> analysis
	Regulatory Status
RUO	Research Use Only
	Manufacturer

15. REFERENCES

1. Yáñez-Mó M, Siljander P, Andreu Z, Bedina Zavec A, Borràs F, Buzas E et al. Biological properties of extracellular vesicles and their physiological functions. Journal of Extracellular Vesicles. 2015;4 (1):27066.
2. Pitt JM, André F, Amigorena S, Soria JC, Eggermont A, Kroemer G, Zitvogel L. Dendritic cell-derived exosomes for cancer therapy. J Clin Invest. 2016.
3. Tkach M, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. 2016 Cell 10;164(6):1226-32.
4. Becker A, Thakur BK, Weiss JM1, Kim HS, Peinado H, Lyden D Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell 2016 Dec 12;30(6):836-848.
5. López-Cobo S, Campos-Silva C, Valés-Gómez M. Glycosyl-Phosphatidyl-Inositol (GPI)-Anchors and Metalloproteases: Their Roles in the Regulation of Exosome Composition and NKG2D-Mediated Immune Recognition. Front Cell Dev Biol. 2016 Sep 12;4:97.
6. Jonathan M. Pitt, Guido Kroemer, Laurence Zitvogel Extracellular vesicles: masters of intercellular communication and potential clinical interventions. 2016 J Clin Invest. 2016;126(4):1139-1143

16. MANUFACTURED BY

IMMUNOSTEP S.L.
Address: Avda. Universidad de Coimbra, s/n
Cancer Research Center (C.I.C)
Campus de Unamuno
37007 Salamanca (Spain)
Telf./fax: (+34) 923 294 827
E-mail: info@immunostep.com
www.immunostep.com