# ExoELISA-Step

## Enzyme Immunoassay for the detection and quantification of Exosomes

| REF | | Σ | |
|---|---|---|---|
| Exo2506 | ELISA assay for detection and quantification of exosomes from cell culture/ urine | 96 test | RUO |
| 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 subsequent washing, an antigen-specific HRP-conjugated antibody is added. After an intermediate wash to remove any excess antibody, a colourimetric reaction mediated by the HRP enzyme is observed with the naked eye when the substrate solution (TMB) is added. This reaction can be quantified using a spectrophotometer or colorimeter. The following protocol describes how a direct ELISA is performed.

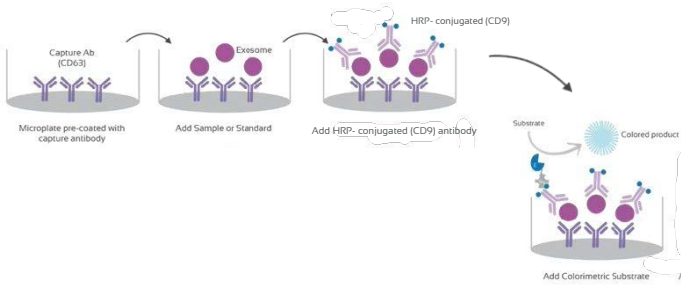



Figure 1: indirect ELISA graphical representation.

## 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.<br>• Ref: Exo2506 Contains PC3 lyophilized exosome standard.<br>• Ref: Exo2508 contains serum/plasma lyophilized exosome standard. | 1 vial (100 µg) |
| Washing buffer 20x | HBS 0,05% Tween washing solution | 50 ml |
| 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 perservative. | 25 ml |
| HRP-Conjugated (100X) | Monoclonal Anti-Human CD9 HRP conjugated ml | 120 ul |
| Substrate solution | TMB | 1 bottle (12 ml) |
| Stop solution | H2SO40,5M | 1 bottle (12 ml) |
| 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 box label if kept at 2-8°C. Do not use after the date indicated.

| DESCRIPTION | PREPARATION | STATE | STORE |
|---|---|---|---|
| Immunoplate | Immunoplate 96-well format | Drief | 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 |
| HRP-Conjugated | Monoclonal Anti-Human CD9 HRP conjugated ml | Liquid | 2/8°C |
| Substrate solution | TMB | Liquid | 2/8°C |
| Stop solution | H2SO4 0,5M | Liquid | 2/8°C |

## 5. REAGENTS NOT PROVIDED

- Calibrated spectrophotometer for Reading ELISA plates at 450 nm and 620 nm.
- Adjustable, calibrated micropipettes covering a range of 1-1000 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. Custo- mer 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 2°C and 8°C for up to 2 years without functional compromise. Immunostep recommends storing small, single –use aliquots of reconstituted exosomes, at – 20°C for up to one month or at – 80°C 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 4°C 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 n°1. 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 n°1 (1/1 dilution) to Eppendorf n°2 (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.

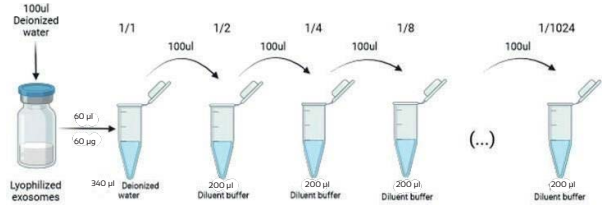
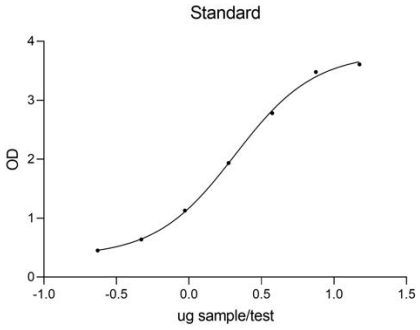



Figure 2: Graphical Representation of the serial dilution of the Standard included in the kit.

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/well (100ul/well) |
|---|---|
| 1 | 15 |
| 2 | 7,5 |
| 3 | 3,75 |
| 4 | 1,875 |
| 5 | 0,9375 |
| 6 | 0,46875 |
| 7 | 0,234375 |
| 8 | 0 |

Figure 3: Four-parameter logistic regression.

## 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.

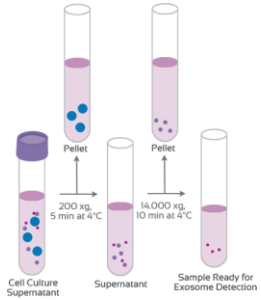



Figure 4: Cell Culture Supernatant pretreatment workflow for direct exosome detection. Please, refer to https://immunostep.com/exosomes/ for additional technical information.

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

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.

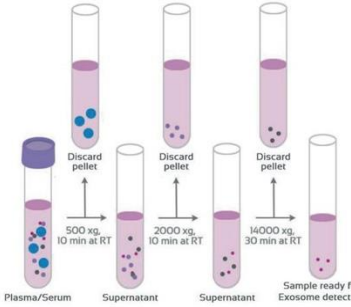



Figure 5: Serum Supernatant pretreatment workflow for direct exosome detection.

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 + 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 3 00 µl of 1X wash buffer for each wash. Leave wash buff er in each we ll for 30-6 0 seconds for each wash cycle. After washing , completely remove all liquid from th e microplate by ta pping it on absorbent paper with the openings facing down to remove all residua l w ash buffer. |
| 3 | Antibodies binding | Add 100ul/well of the antibody dilution (1:100 in sample diluent). Seal the plate with parafilm and incubate an hour at +37°C. |
| 4 | Washing | If necessary, remo ve th e prot ective fo il. Empty th e wells and wash as described previously (st ep 2). |
| 5 | 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. |
| 6 | Stopping | Add 100 µl of the stop solution (1X - ready to use) to each well, trying to follow the same orde r in which the substrate solution was added. |
| 7 | Absorbance measurement | Measure the optical densities (O.D.) of each well on a microplate spectrophotome ter at 450 nm , with in 30 minutes of adding the Stop Solution.<br>Before measurement, carefully shake the plate to ensure a homogeneous distribution of th e 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 |
| | Description |
| RUO | Research Use Only |
| | Manufacturer |

## 15. REFERENCES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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. Thach 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.
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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


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