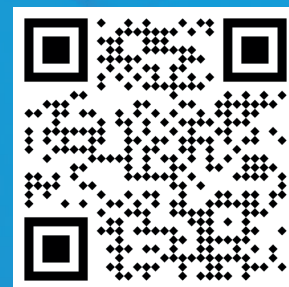


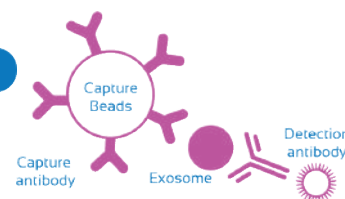
Exosome Products For Research and Diagnosis



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Human Exosome Detection Kits

Still Performing WB and NTA for detection and characterization of exosomes?
Discover the **most powerful reagents for exosome detection by flow cytometry.**

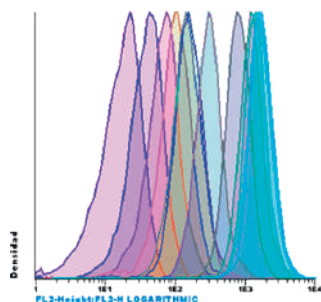


The kit is a simple immunobead assay for isolation/detection of exosome, using a bead-bound capture antibody and a fluorochrome conjugated detection antibody. The kit provides reproducible results and can be run in parallel to exosome immunophenotyping.

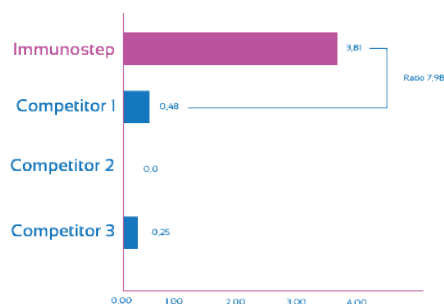
Immunostep's ExoStep⁽¹⁾, is intended for the immunoisolation (immunomagnetic or FACS) and Flow Cytometry analysis of pre-enriched human exosomes from biofluids (plasma, serum, urine) or cell culture media.

Main Characteristics of Exostep

- 1 Specific and unambiguous detection
- 2 Quantitative analysis, excellent correlation between fluorescence and the amount of exosomes
- 3 Direct detection of Exosomes in cell culture supernatant and biological fluids. Without isolation or precipitation
- 4 Very small amount of sample needed
- 5 Greater sensitivity, wide dynamic range. Guaranteed detection even with small sample quantities



Exostep cytometric histogram showing the dynamic range (0-128µg) of exosomes from PC3 Cell line using ExoStep.

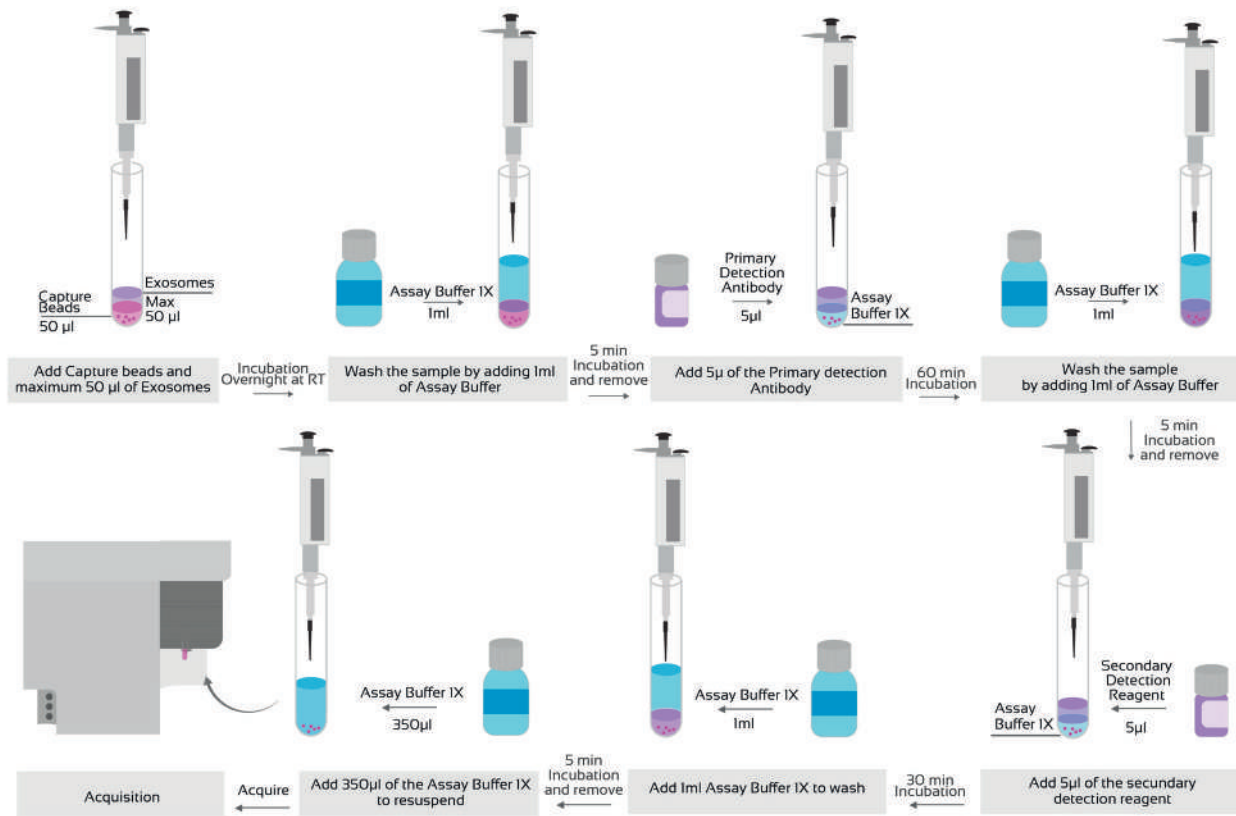


Sensitivity comparing among competitors

- 6 Reproducible
- 7 Allowing simultaneous immunophenotyping of exosomes capture population

(1) This kit was developed as part of a collaboration project between Immunostep, National Centre for Biotechnology, centre that forms part of the Spanish National Research Council (CNB-CSIC) and Fundación de la Universidad Autónoma de Madrid.

ExoStep Protocol- General Kit



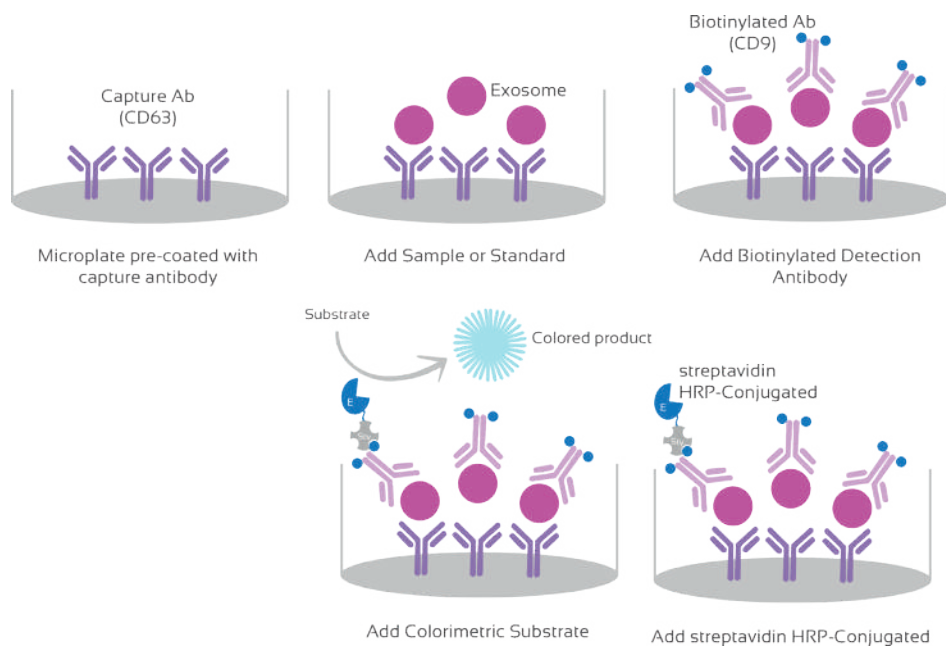
| Name | Unit size | Reference | Content of the Kit | Intended Use |
|-----------------------------|-----------|---------------------------|---|---|
| Exostep™ Culture | 25 tests | ExoS-25-C9 | Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer IOX | This kit is intended for RUO in the detection of human exosomes from cell culture samples |
| Exostep™ Plasma | 25 tests | ExoS-25-P81 | Superparamagnetic Capture Beads (CD9 Capture Beads) Primary detection antibody (CD81 PE) Assay Buffer IOX | This kit is intended for RUO in the detection of human exosomes from Plasma samples |
| Exostep™ Urine | 25 tests | ExoS-25-U9 | Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer IOX | This kit is intended for RUO in the detection of human exosomes from Urine samples |
| Exostep™ Culture + Standard | 50 tests | ExoS-50-CST9 | Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer IOX Lyophilized exosomes (1x10 ¹²) from PC-3 Human prostate cancer | This kit is intended for RUO in the detection of human exosomes from Cell Culture samples |
| Exostep™ Plasma + Standard | 50 tests | ExoS-50-PST81 | Superparamagnetic Capture Beads (CD9 Capture Beads) Primary detection antibody (CD81 PE) Assay Buffer IOX Lyophilized exosomes (1x10 ¹²) from Human Serum | This kit is intended for RUO in the detection of human exosomes from Plasma samples |
| Exostep™ General Kit | 25 tests | ExoS-25-G9 ExoS-25-G81 | Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 Biotin) or (CD81 Biotin) Secondary detection reagent (PE Conjugated) Assay Buffer IOX | This kit is intended for RUO in the detection of human exosomes from cell culture supernatant and biological fluids |

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. Campos S, Suárez H, Jara-Acevedo R, Linares-Espinós E, Martínez-Piñeiro L, Yáñez-Mó M, Valés-Gómez M. High sensitivity detection of extracellular vesicles immune-captured from urine by conventional flow cytometry. *Sci Rep*. 2019; Feb 14;9(1):2042.

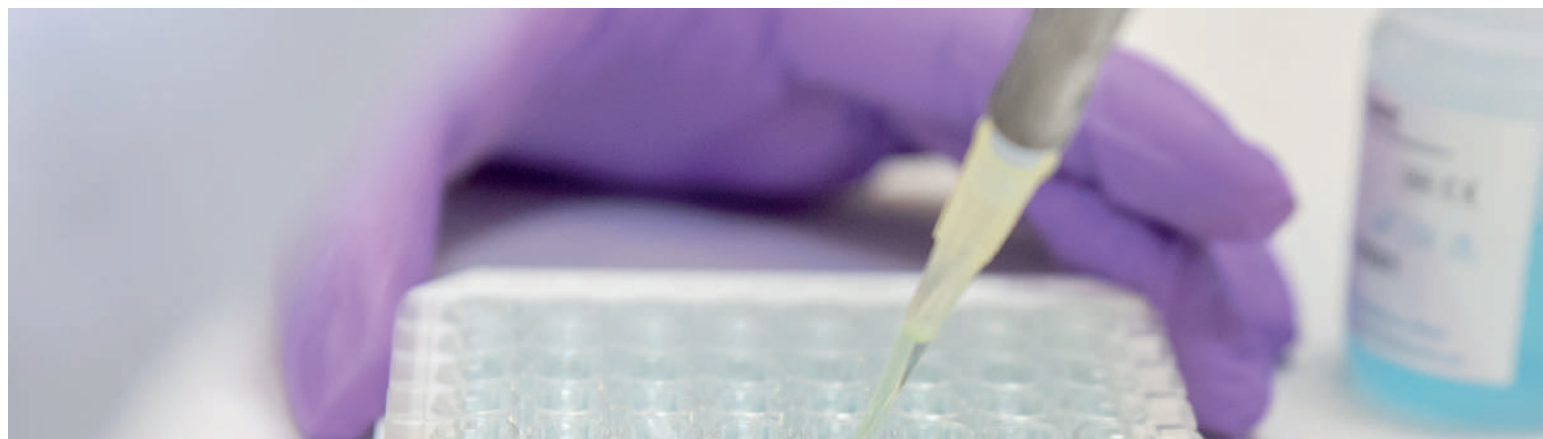
Based on original ExoStep, Immunostep introduces ExoELISA-Step kit. This kit is intended for the detection and quantification of exosomes by ELISA, which is an easy to perform and commonly used method.

Main Characteristics of ExoELISA-step

- 1 Direct detection of Exosomes in cell culture supernatant and biological fluids
- 2 Without isolation or precipitation
- 3 Successfully detecting and quantifying exosomes



| Name | Unit size | Reference | Content of the Kit | Intended Use |
|----------------------------|-----------|-----------|---|---|
| ExoELISA-Step Serum/Plasma | 96 tests | EXO2508 | Standard (Serum/plasma) washing buffer, buffer diluent, primary antibody (CD9 biotin) y, HRP-Conjugated, substrate solution, stop solution and immunoplate and sealing film | This kit is intended for RUO in the detection of human exosomes from Plasma samples |
| ExoELISA-Step Cell Culture | 96 tests | EXO2506 | Standard for assay calibration (PC3), washing buffer, buffer diluent, primary antibody (CD9 biotin) y, HRP-Conjugated, substrate solution, stop solution and immunoplate and sealing film | This kit is intended for RUO in the detection of human exosomes from cell culture samples |





Human Capture Beads

Specific exosomes purification from one cell type or exosome subpopulation characterization remains a challenge. Thanks to our human capture beads, it is possible to isolate specific exosomes among others, from biological fluids (serum, plasmas, CSF, saliva, urine, etc.) without previous sample enrichment procedures.

Main Characteristics of Human Capture Beads

- 1 Allows the detection of isolated exosomes from different isolation techniques, such as differential ultracentrifugation, precipitation solutions or size exclusion chromatography columns
- 2 Direct detection in the sample without the need for ultracentrifugation
- 3 Compatible with downstream analysis (WB, mRNA, miRNA, etc.)

| Product Description | Reference | Unit Size |
|--|--------------|-----------|
| Human CD9 Capture Beads for Flow Detection | 9CB-25 | 25 test |
| Human CD63 Capture Beads for Flow Detection | 63CB-25 | 25 test |
| Human CD81 Capture Beads for Flow Detection | 81CB-25 | 25 test |
| Human CD326 (EpCAM) Capture Beads for Flow Detection | 326CB-25 | 25 test |
| Human CD274 (PD-L1) Capture Beads for Flow Detection | 274CB-25 | 25 test |
| Human IgG1 Capture Beads (Isotype Control) for Flow Detection | IGG1CB-25 | 25 test |
| Human IgG2a Capture Beads (Isotype Control) for Flow Detection | ICIGG2ACB-25 | 25 test |



Mouse Exosome Detection Kits

Based on original ExoStep, Immunostep introduces its rodent ExoStep. This kit is intended for the flow cytometry analysis of pre-enriched CD63+ rodent exosomes from cell culture media and biological fluids.

- 1 Direct detection of Exosomes in cell culture supernatant and biological fluids
- 2 Without isolation or precipitation
- 3 Reproducible greater sensitivity

| Name | Unit size | Reference | Content of the Kit | Intended Use |
|-------------------------------------|-----------|--------------------------|--|---|
| Mouse Exostep TM Culture | 25 tests | MO2ExoS-25-C | Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 Biotin) Secondary detection reagent (PE Conjugated) Assay Buffer 10X | This kit is intended for RUO in the detection of mouse exosomes from Cell Culture samples |
| Anti-Mouse CD9 Antibody | 25 tests | MO9BExo-25 MO9FExo-25 | Biotin rat anti-mouse CD9 Antibody FITC rat anti-mouse CD9 Antibody | |
| Mouse CD63 Capture | 25 tests | MO63CB-25 | Beads for flow detection | |

Custom-Made Immunobeads

Immunostep provides custom-made beads for research, academic or industrial needs. Just choose your coating antibody for exosomes capture and we will perform all the conjugation and validation. Get further details at: <https://www.immunostep.com>



Human Exosomal Antibody Markers

Antibodies are an essential tool for scientists in biomedical and diagnostic research. Antibodies targeted against exosome associated antigens (exosome marker antibodies: CD63, CD9, CD81, etc.) facilitate the characterization and/or quantification of exosomes in cells, tissues or other biological samples.

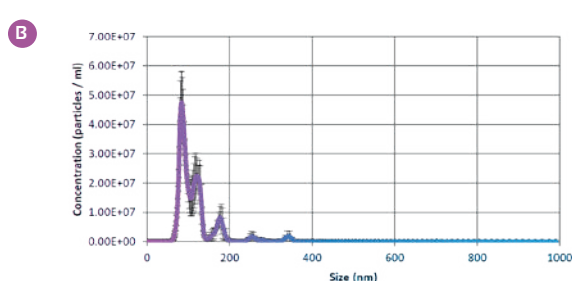
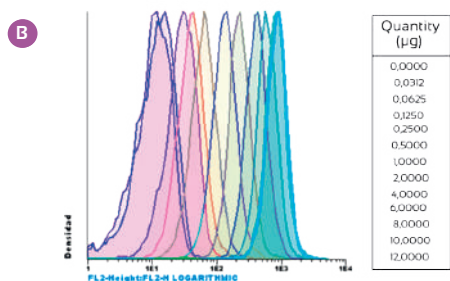
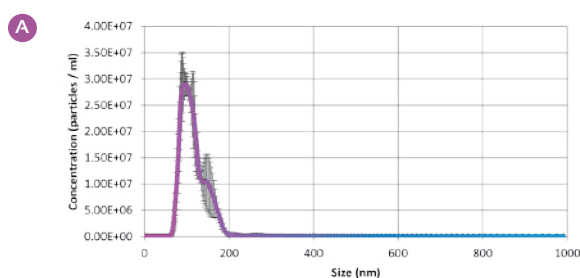
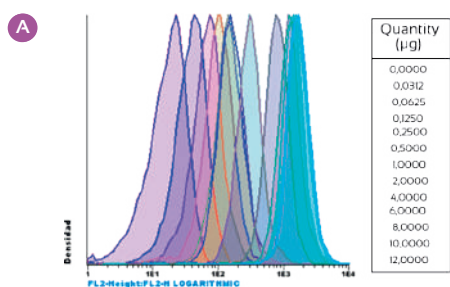
Immunostep offers a **wide range and high quality multi-assay/species validated antibodies** for various exosome markers. These antibodies are available in several different conjugated formats, with the right formulation for exosome detection. Besides, these antibodies can be used together with our Exostep Detection kit for exosome subpopulations characterization

| Product Description | Reference | Unit |
|---|--------------|-------|
| Biotin Mouse anti-human CD63 Antibody | 63BExo-25 | 25 µg |
| FITC Mouse anti-human CD63 Antibody | 63FExo-25 | 25 µg |
| PE Mouse anti-human CD63 Antibody | 63PEExo-25 | 25 µg |
| CF-Blue Mouse anti-human CD63 Antibody | 63CFBEExo-25 | 25 µg |
| Biotin mouse anti-human CD9 Antibody | 9BExo-25 | 25 µg |
| CF-Blue Mouse anti-human CD9 Antibody | 9CFExo-25 | 25 µg |
| FITC mouse anti-human CD9 Antibody | 9FExo-25 | 25 µg |
| PE mouse anti-human CD9 Antibody | 9PEExo-25 | 25 µg |
| PE mouse anti-human CD81 Antibody | 81PEExo-25 | 25 µg |
| Biotin mouse anti- human CD326 Antibody | 326BExo-25 | 25 µg |
| FITC mouse anti-human CD326 Antibody | 326FExo-25 | 25 µg |
| PE mouse anti-human CD326 Antibody | 326PEExo-25 | 25 µg |
| Biotin mouse anti-human CD81 Antibody | 81BExo-25 | 25 µg |
| CF-Blue mouse anti-human CD81 Antibody | 81CFExo-25 | 25 µg |
| FITC mouse anti-human CD81 Antibody | 81FExo-25 | 25 µg |



Lyophilized Exosome Standards

The highest pure Lyophilized Exosome Standards from human biofluids (plasma, serum) and different cell culture media. Immunostep lyophilized standards have been validated by WB and FCM, for overall protein content and particle number by Nanoparticles Tracking Analysis (NTA).



Dynamic range of fresh **A** and lyophilized **B** PC3 exosomes analyzed by flow cytometry. Relationship between background noise and specific signal at different exosome concentrations. Exosomes were captured by CD63+ (Clone TEA3/18) capture beads and subsequently detected by Anti-CD9 PE (Clone VJI/20).

Exosome analysis and comparative of fresh **A** and lyophilized **B** plasma exosomes for particle size and concentration by NTA, NanoSight LM10HSB. Analysis was carried out with 1 µl of purified exosomes diluted in 999 µl of HEPES buffer (dilution 1:1000). The purified exosomes showed a size distribution profiles, with peak diameters from 50 – 150 nm and concentrations about 1x10¹⁰ exosomes/ml.

- 1 Highly pure exosomes, providing better performance than competitors
- 2 Guaranteed stability thanks to an exclusive lyophilization procedure
- 3 Exhaustive validation batch to batch, by WB, NTA, cytometry and functional analysis in vitro
- 4 Tested in application for Medicine Regenerative, Skin, dermatological and pharma companies
- 5 miRNA content of our exosome cell lines provided

| Product Description | Reference | Unit Size |
|--|-----------|-----------|
| Exosomes from PC-3, a human metastatic prostate cancer cell line | ExoPC3 | 100 µg |
| Exosome from HT-29, a human colon cancer cell line | ExoHT29 | 100 µg |
| Exosome from MCF-7, a human breast cancer cell line | ExoMCF7 | 100 µg |
| Exosome from Serum, human Serum | ExoSERUM | 100 µg |
| Exosomes from A-375, a human malignant melanoma cell line | ExoA375 | 100 µg |
| Adipose-derived Mesenchymal stem/stromal cells (MSCs) derived exosomes | ExoMSC | 100 µg |
| Exosomes from RPMI, a human myeloma cell line | ExoRPMI | 100 µg |
| Exosomes from CaCo2, a human colon cancer cell line | ExoCaCo2 | 100 µg |
| Exosomes from A-549, a human lung cancer cell line | ExoA549 | 100 µg |
| Exosomes from PANC-1, a human pancreas cancer cell line | ExoPANC1 | 100 µg |

Custom-Made Standards

Immunostep provides custom-made standards for research, academic or industrial. Get further details at: <https://www.immunostep.com>



MOST POPULAR ISOLATION TECHNIQUES

The biological characterization of exosomes requires in most cases the isolation of intact exosomes. In this sense, a large number of methods have been developed for the isolation of exosomes from biological fluids, among which are ultracentrifugation, chromatography, filtration, immunological separation and polymer-based precipitation. Each one of these methods presents its advantages and disadvantages, being the duration of the method, the need to have specialized equipment, the volume of sample, the purity and the low recovery, some of the disadvantages that these methods present.

IMMUNOSTEP offers two of the most common techniques for exosomes isolation with all the guarantees:



Exosome Precipitation Solution

Immunostep's Exosome Precipitation Solution, is intended for the extracellular vesicles (EVs) and specifically exosomes (~50-150 nm) from cell culture media and biofluids (plasma, serum, urine).

Main Characteristics of Exosome Precipitation Solution

- 1 Easy & rapid precipitation solution. Ultracentrifugation free method
- 2 Very clean & better exosomes preparation, reduces carry-over of albumins and immunoglobulins compared to other methods
- 3 Obtain intact exosome suitable for a great variety of protein-sensitive applications and downstream uses
- 4 Increase biomarker sensitivity detection

| Product Description | Reference | Sample | Unit Size |
|---|-----------|---------------------------|-----------|
| Exostep Solution | EPStep | Cell Culture Media, Urine | 12 ml |
| Exosome precipitation from plasma and serum | EPStep-PS | Plasma, Serum | 5 ml |
| Exosome precipitation from Plasma + Trombin | EPStep-T | Plasma | 5 ml |



Exosome Solutions & Buffers

Improve your research with our exclusive solutions & buffers.

| Product Description | Reference | Information | Unit Size |
|-----------------------------|-----------|---|-----------|
| Exosome Detachment Solution | EDSTEP | Designed to elute the exosomes from the antibody-bead complex and allow downstream analysis without interferences | 10 ml |
| Wash Buffer Solution (IOX) | IMS0515 | Contains 10 % albumin in 10mM sodium phosphate, 150 mM NaCl, pH 7.4, and KATHON anti-microbial agent | 20 ml |

Immunostep provides custom-made reagents for research, academic or industrial. Get further details at: <https://www.immunostep.com>



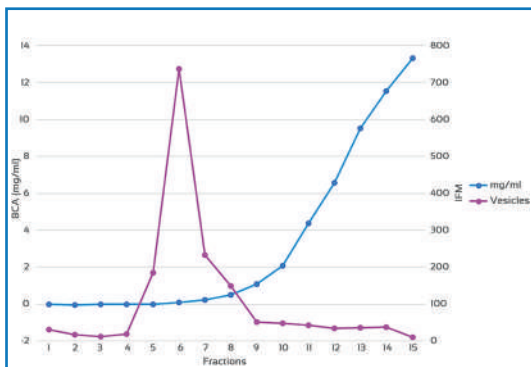
Exosome Isolation Columns

Size exclusion chromatography (SEC) has been described as most efficient method for isolating EVs from complex biological fluids by single-step, with a good recovery and with almost complete removal of contaminants, such as proteins and lipoproteins. Immunostep has developed 70nm and 35nm SEC columns for EVs isolation from complex biological fluids such as: plasma, serum, urine and cerebral spinal fluid.

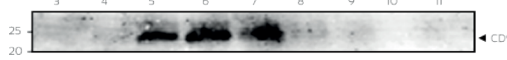
Main Characteristics of Exosome Isolation Columns

- 1 Save Time: easy, and rapid method
- 2 High purity: protein removal & HDL purification
- 3 Excellent recovery: method that maximices recovery (>50%)
- 4 No aggregation: method that reduces the risk of protein complex formation and vesicle aggregation
- 5 Indicated for low volumens
- 6 Standarisable & reproducible
- 7 Protocol compatible with RNAs extraction and RT-PCR

Elution Profile

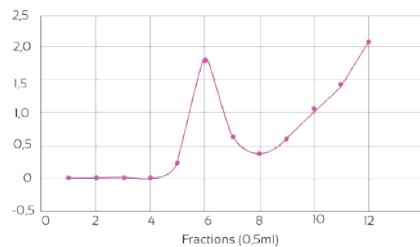


Amount of EVs and protein in each fraction from the column. Comparative of protein (BCA) vs vesicles (FACS CD63+/CD9+) content.

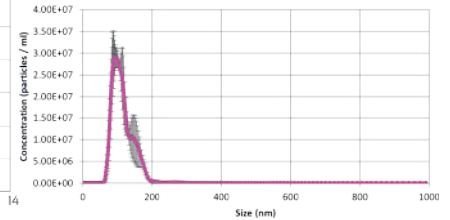


Western Blot. SEC fractions were loaded on SD-PAGE and immunoblotted for CD9 tetraspanin with anti-CD9 (VJI/20), under no-reducing conditions

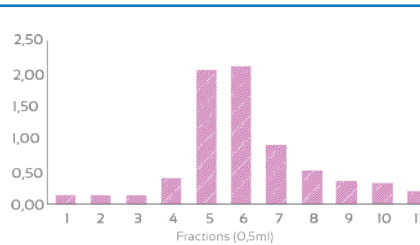
Exosome Plasma isolated by SEC



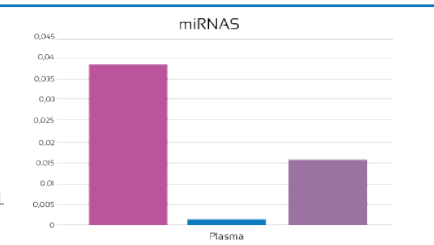
EVs isolation from plasma sample. Elution profile monitored by abs (280).



NanoSight analysis of the EVs recovered from Plasma by SEC columns.



Flow cytometric Analysis of elution fractions. Stain Index = (MFI Positive-MFI background) / 2σ background



Exosomal miRNA levels by quantitative RT-PCR.

Product Description

Reference

Unit Size

| | | |
|-----------------------|-----------|-----------|
| EVs SEC 70nm - 4 pack | SEC7012-4 | 4 Columns |
| EVs SEC 70nm - 8 pack | SEC7012-8 | 8 Columns |
| EVs SEC 35nm - 4 pack | SEC3512-4 | 4 Columns |
| EVs SEC 35nm - 8 pack | SEC3512-8 | 8 Columns |