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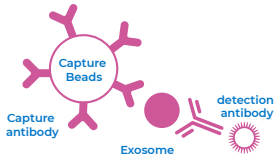
PRODUCT RANGE OF EXOSOME RESEARCH

HUMAN EXOSOME DETECTION KITS EXOSTEP



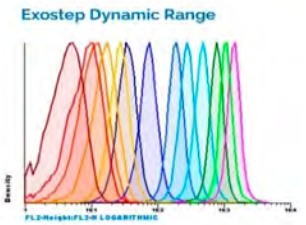
Still performing WB and NTA for detection and characterization of exosomes? Discover the most powerful reagents for exosome detection by flow cytometry

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

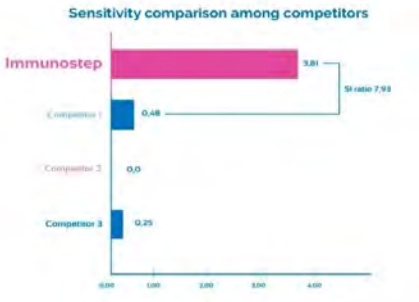
Immunostep's ExoStep[1], is intended for Flow Cytometry analysis of pre-enriched human exosomes from biofluids (plasma, serum, urine) or cell culture media

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



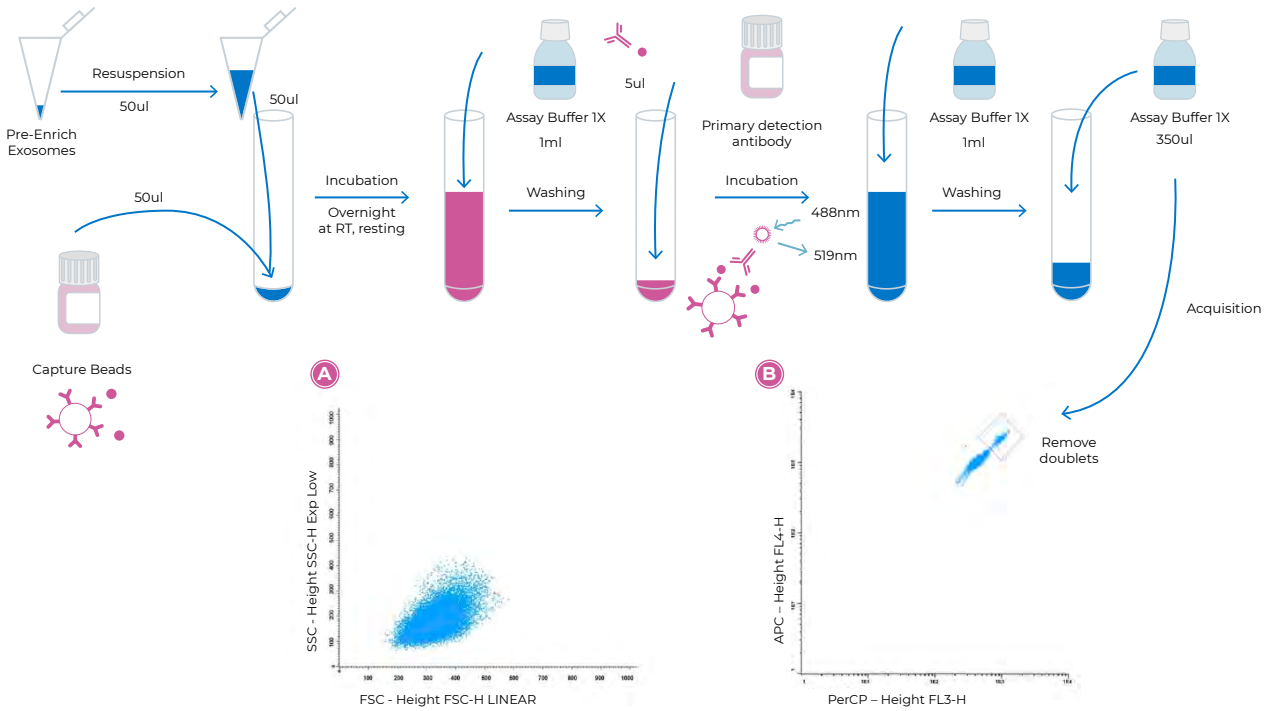
Flow cytometry histogram showing the dynamic range (0-128 µg) of exosomes from PC_3 Cell Line using ExoStep



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 Biotechnolgy, centre that forms part of the Spanish National Research Council (CNB-CSIC) and Fundación de la Universidad Autonoma de Madrid.

EXOSTEP PROTOCOL



Dot-plot gating strategy for acquisition and analysis. FSC vs SSC (A) and PerCP vs APC (B).

NAME	UNIT SIZE	REFERENCE	CONTENT OF THE KIT	INTENDED USE
ExoStep™ Culture	25 test	ExoS-25-C9	Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer 10X	This kit is intended for RUO in the detection of human exosomes from Cell Culture Samples
ExoStep™ Plasma	25 test	ExoS-25-P81	Superparamagnetic Capture Beads (CD9 Capture Beads) Primary detection antibody (CD81 PE) Assay Buffer 10X	This kit is intended for RUO in the detection of human exosomes from Plasma Samples
ExoStep™ Urine	25 test	ExoS-25-U9	Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer 10X	This kit is intended for RUO in the detection of human exosomes from Urine Samples
ExoStep™ Culture + Standard	25 test	ExoS-25-CST9	Superparamagnetic Capture Beads (CD63 Capture Beads) Primary detection antibody (CD9 PE) Assay Buffer 10X Lyophilized exosomes (1x10^12) from PC-3 Human prostate cancer	This kit is intended for RUO in the quantitative detection of human exosomes from Cell Culture Samples
ExoStep™ Plasma + Standard	25 test	ExoS-25-PST81	Superparamagnetic Capture Beads (CD9 Capture Beads) Primary detection antibody (CD81 PE) Assay Buffer 10X Lyophilized exosomes (1x10^12) from Human Plasma	This kit is intended for RUO in the quantitative detection of human exosomes from Plasma Samples
ExoStep™ General Kit	25 test	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 10X	This kit is intended for RUO in the detection of human exosomes from Cell Culture, Plasma or Urine Samples

1.- Campos S, Suárez H, Jara-Acevedo R, Linares-Espinós E, Martínez-Piñeiro L, Yáñez-Mó M, Valés-Gómez M. High sensitivity detection of extracelular vesicles immune-captured from urine by conventional flow citometry. Sci Rep. 2019; Feb 14;9(1):2042.
2.- Jara-Acevedo R, Campos-Silva C, Valés-Gómez M, YáñezMó M, Suárez H, Fuentes M. Exosome beads array for multiplexed phenotyping in cancer. J Proteomics. 2019; Apr 30;198:87-97

HUMAN CAPTURE BEADS

Specific exosomes purification from one cell type, or exosome subpopulationcharacterization, remains a challenge.

Thanks to our human capture beads, it is possible to isolate specific exosomes among others, from biological fluids (serum, plasma, CSF, saliva, urine, etc.) without previous sample enrichment procedures.

Main Characteristics of Human Capture Beads

1. Allows specific exosome isolation freom different purification techniques, such as differential ultracentrifugation, precipitation solutions or size exclusion chromatography columns
2. Direct specific exosome isolation without previous enrichment
3. Fully compatible with downstream analysis (WB, mRNA, miRNA, etc)

PRODUCT DESCRIPTION	REFERENCE	UNIT SIZE
Human IgG1 Capture Beads (Isotype Control) for Flow Detection	IGG1CB-25	25 test
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

MOUSE EXOSOME DETECTION KITS

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

Main Characteristics of Mouse Exostep Detection

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

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 perfom all the conjugation and validation. Get further details at: ***https://www.immunostep.com/content/40-exosomes-service***

NAME	UNIT SIZE	REFERENCE	CONTENT OF THE KIT	INTENDED USE
Mouse ExoStep™ Culture	25 test	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 test 25 test	MO9BExo-25 MO9FExo-25	Biotin rat anti-mouse CD9 Antibody FITC rat anti-mouse CD9 Antibody	
Mouse CD63 Capture	25 test	MO63CB-25	Beads for Flow Detection	

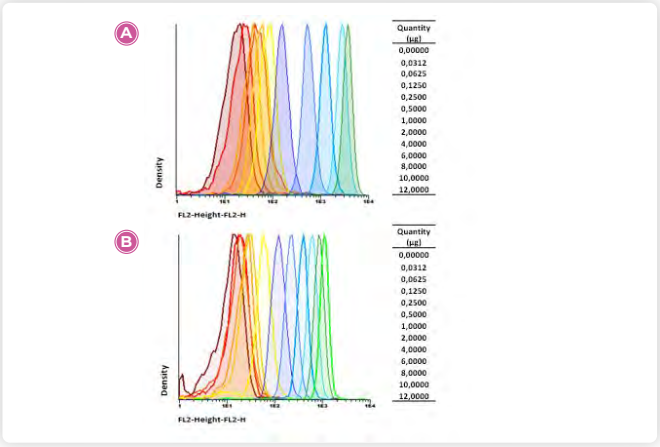
HUMAN EXOSOMAL ANTIBODY MARKERS

Antibodies are an essential tool for scientists in biomedical and diagnostic research. Antibodies targeted against exosome associated antigens 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 exosomal markers. These antibodies are available in several different conjugated formats, with the right formulation for exosome detection. Besides, these antibodies can be used in combination with our Exostep Detection kit for exosome subpopulations characterization

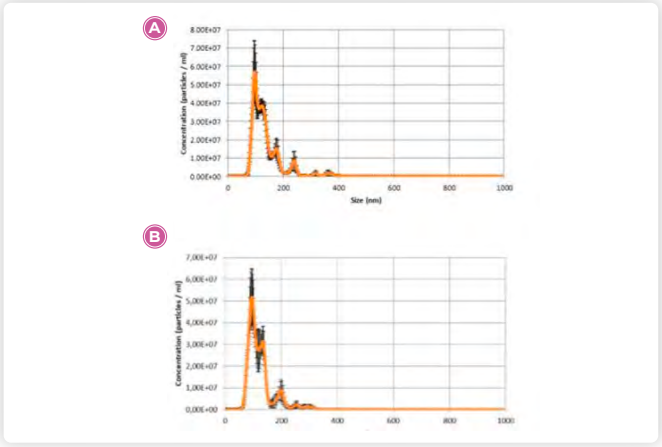
PRODUCT DESCRIPTION	REFERENCE	UNIT SIZE
Biotin mouse anti-human CD63 Antibody	63BExo-25	25 µg
FITC Mouse anti-human CD63 Antibody	63FExo-25	25 µg
PE Mouse anti-human CD63 Antibody	63PEExo-25	25 µg
CF-Blue Mouse anti-human CD63 Antibody	63CFBEExo-25	25 µg
Biotin mouse anti-human CD9 Antibody	9BExo-25	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) 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 VJ1/20).



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 (diution 1:1000). The purified exosomes showed a size distribution profiles, with peak diameters from 50 – 150 nm and concentrations about 1x1010 exosomes/ml.

Main Characteristics of Lyophilized Exosome Standards

1. High 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

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 Plasma, a human breast cancer cell lin	ExoPLASMA	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

Custom-Made Standards
Immunostep provides custom-made standards for research, academic or industrial. Get further details at:
https://www.immunostep.com/content/40-exosomes-service

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 preparations. Reduces carry-over of albumins and immunoglobulins compared to other isolation methods
3. Obtain intact exosomes suitable for a great variety of protein-senstitive applications and downstream uses
4. Increase biomarker sensivity detection

PRODUCT DESCRIPTION	REFERENCE	SAMPLE	VOL
Exosome Solution	EPStep	Cell Culture media, Urine	12ml
Exosome precipitation from plasma and serum	EPStep-PS	Plasma, serum	5ml
Exosome precipitation from Plasma + Trombin	EPStep-T	Plasma	5ml

EXOSOME ISOLATION COLUMNS

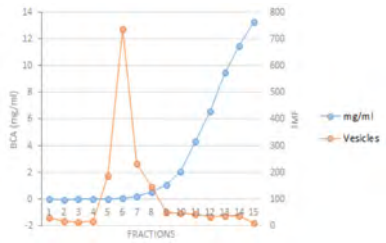
Size exclusion chromatography (SEC) has been described as most efficient method for isolating EVs from complex biological fluids by single-step, with a good recovery and with almost complete removal of contaminants, such as proteins and lipoproteins

Immunostep has developed **70nm** and **35nm** SEC columns for EVs isolation from complex biological fluids such as: plasma, serum, urine and cerebral spinal fluid.

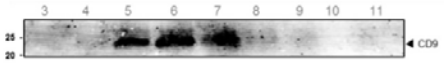
Main Characteristics of Exosome Isolation Columns

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



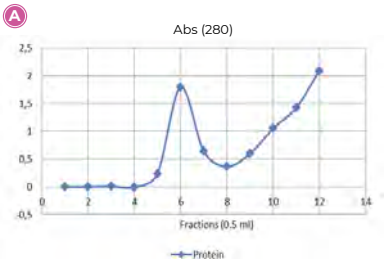
Amount of EVs and protein in each fraction from the column. Comparative of protein (BCA) vs vesicles (FACS CD63+/CD9+) content.



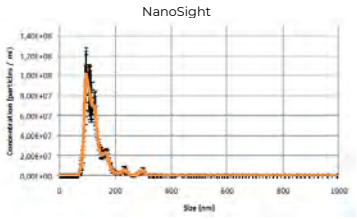
Western Blot. SEC fractions were loaded on SDS-PAGE and immunoblotted for CD9 tetraspanin with anti-CD9 (VJ1/20), under non-reducing conditions.

Exosome Plasma isolated by SEC

A

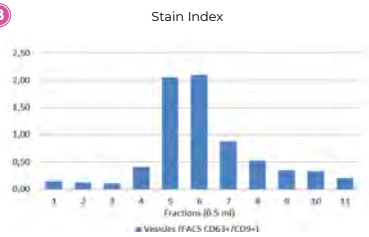


EVs isolation from plasma sample. Elution profile monitored by Abs (280).

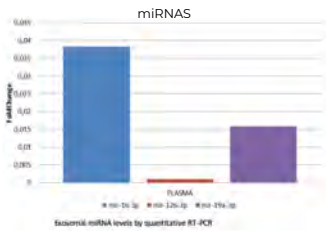


NanoSight analysis of the EVs recovered from Plasma by SEC columns.

B



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 70 nm - 4 Pack	SEC7012-4	4 Columns
Evs SEC 70 nm - 8 Pack	SEC7012-8	8 Columns
Evs SEC 35 nm - 4 Pack	SEC3512-4	4 Columns
Evs SEC 35 nm - 8 Pack	SEC3512-8	8 Columns

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