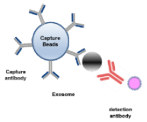
we know how
immunostep

Flow cytometry methods and kits for the isolation and characterization of EVs

Our Portfolio

Human Exosome Detection Kits

Mouse Exosome Detection Kits



Capture Beads

Exosome precipitation solution

Lyophilized Exosome Standards

Antibodies

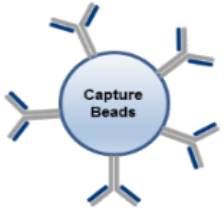
Secondary Antibodies

Exosome Isolation Columns

MOST POPULAR ISOLATION TECHNIQUES

Differential Ultracentrifugation

Precipitation Solutions



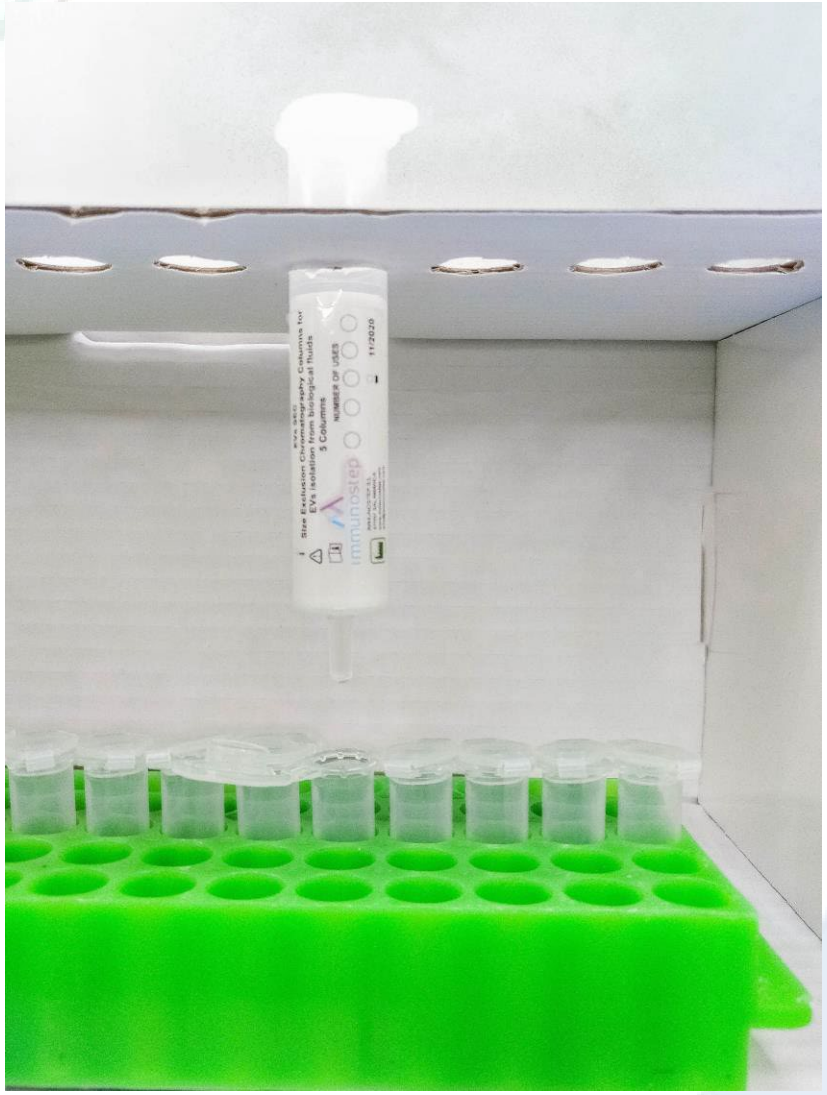
ImmunoCapture

Size Exclusion Chromatography Columns

SPECIFICATIONS	
Separation size	70-1000nm
Volumetric Flow Rate at RT	<0.5 ml/min
Sample volume	<1 ml (500 µl optimal volume)
Column volume	10 ml
Void volume	3 ml
pH stability working range	3-13
pH stability cleaning in place (CIP)	2-14
Shelf life	12 months at 2-8ºC

- ✓ Indicated for low volumns
- ✓ Rapid & Reliable Exosome Purification
- ✓ Standardisable & Reproducible
- ✓ Pure & Clean Isolated Samples

Size Exclusion Chromatography Columns



Preparation for use

1. Place the column in a holder and level it, make sure the column is vertical.
2. Remove the botton cap.
3. Leave the top-cap carefully.

Column equilibration

1. Rinse the column with 7 mL of elution buffer (PBS with sodium citrate).
2. Make sure enough time is given for the column to be in the operational temperature range.
3. Do not allow the column to run dry. The top filter must stay wet.
4. Use only fresh filtered (0.2 µL) buffer to avoid particulate contamination.

Sample fraction collection

1. When the column is used according to the protocol, for 500µl fractions, the first four fractions (2.0 mL), is the void volume which does not contain vesicles, and they elute predominantly in fractions 5, 6 and 7 with removal of protein contamination. Fractions beyond 8 usually contain higher protein and lower vesicle levels (Fig 1).

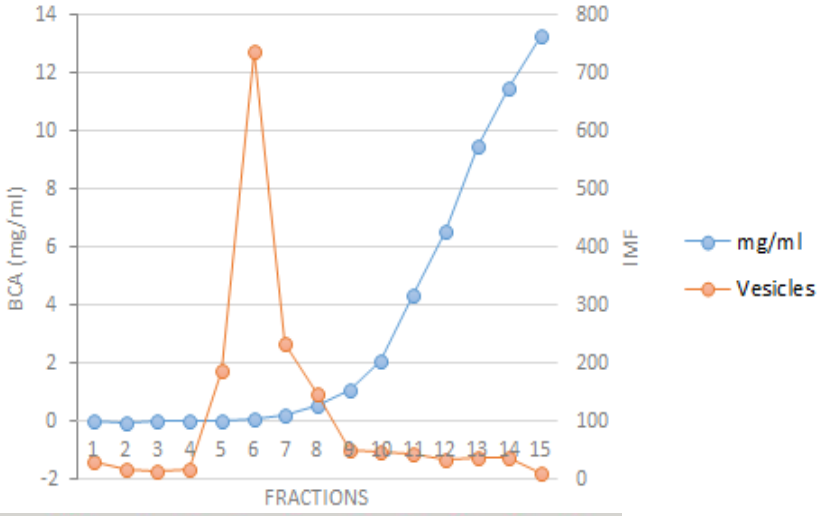
Post collection of vesicles

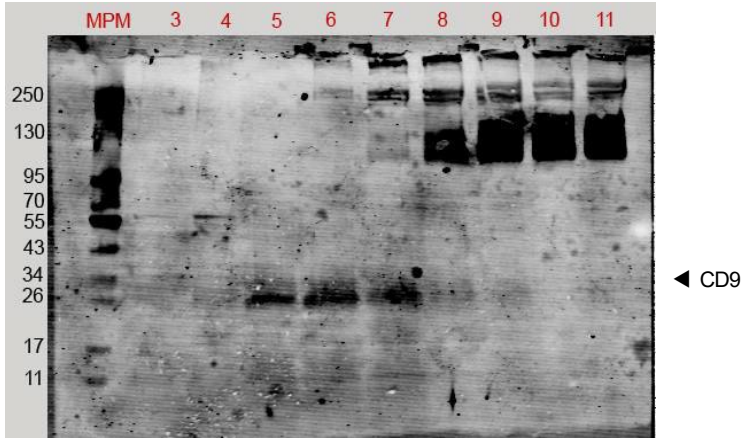
1. After the collection of the vesicle fractions, flush the column with 14 to 21 mL of equilibration buffer to flush out all the protein and small molecules before the next sample application. After that, flush the column with 7 mL of a bacteriostatic agent and store as indicated in the storage section

.

SEC columns Performance

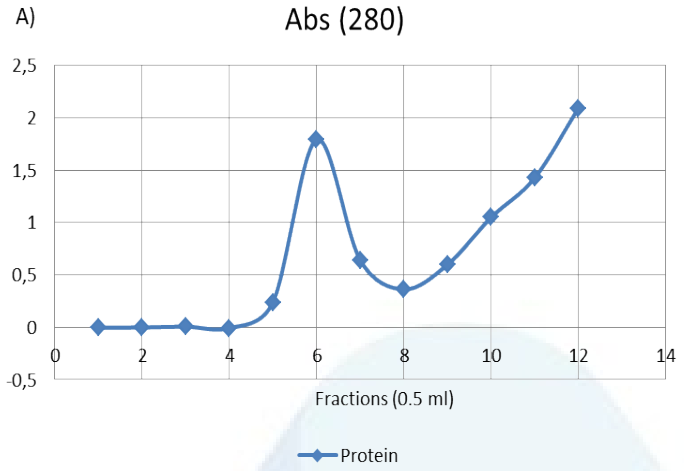
Elution profile

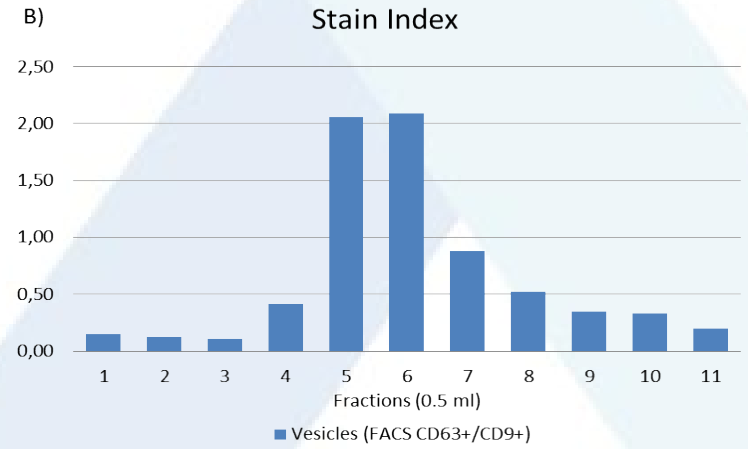




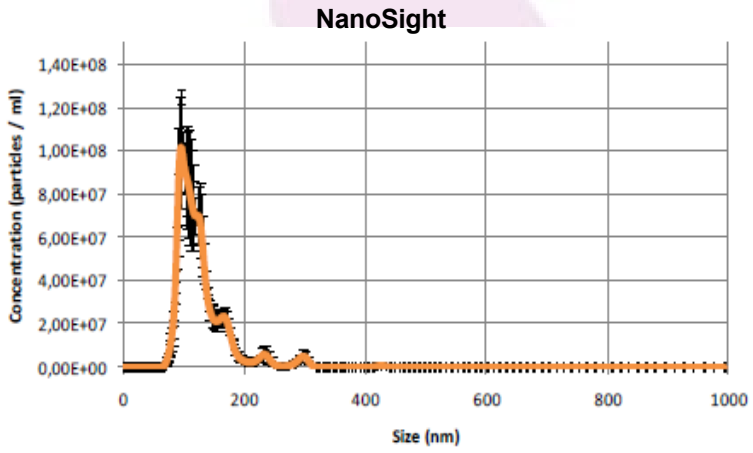
◄ CD9

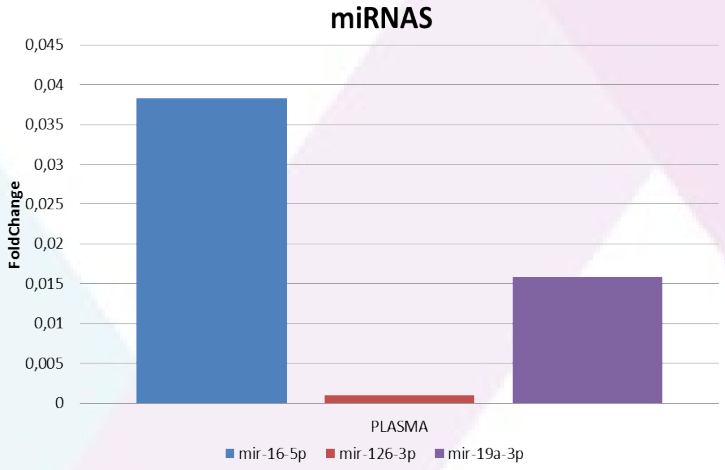
Exosome Plasma isolated by SEC





Stain index: (MFI positive- MFI background)/ 2σ background)

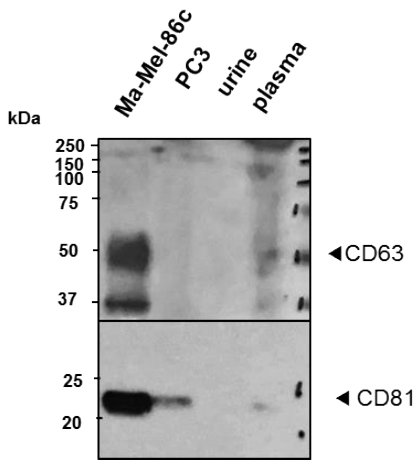




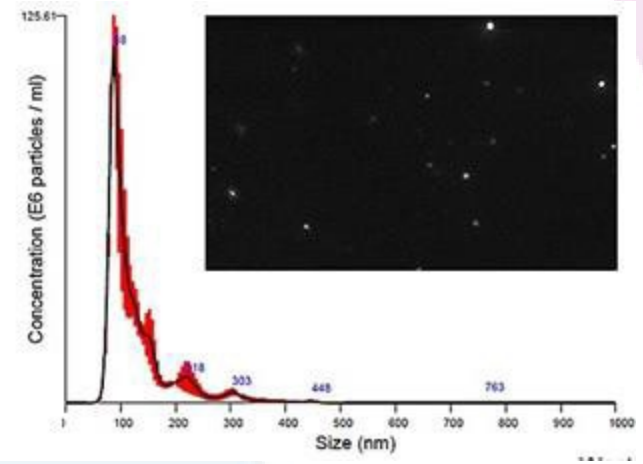
Exosomal miRNA levels by quantitative RT-PCR

Common Methods to Analyze EVs Proteins

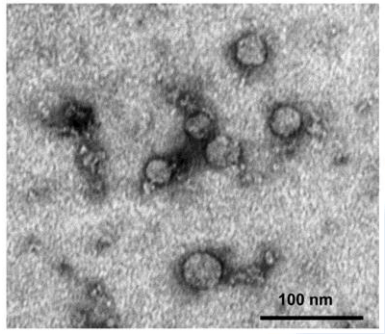
Western Blot



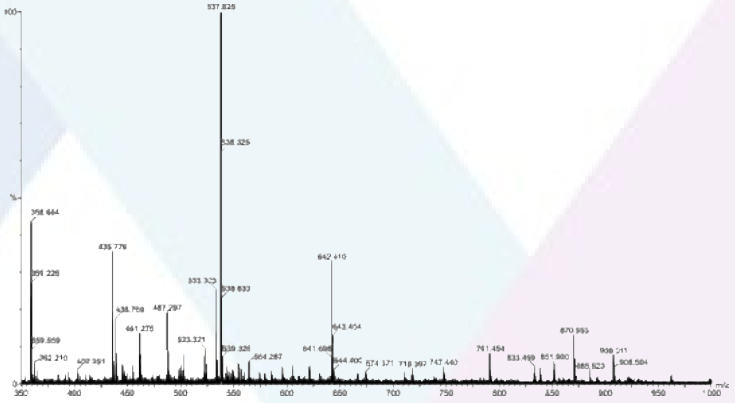
Nanoparticle Tracking Analysis



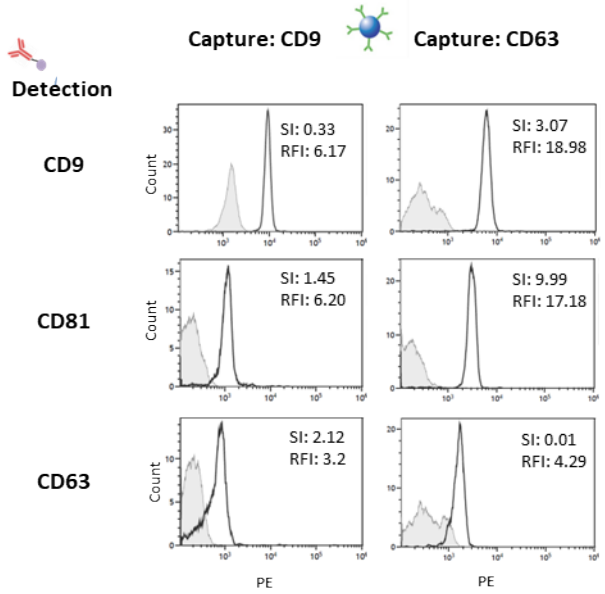
Electron microscopy



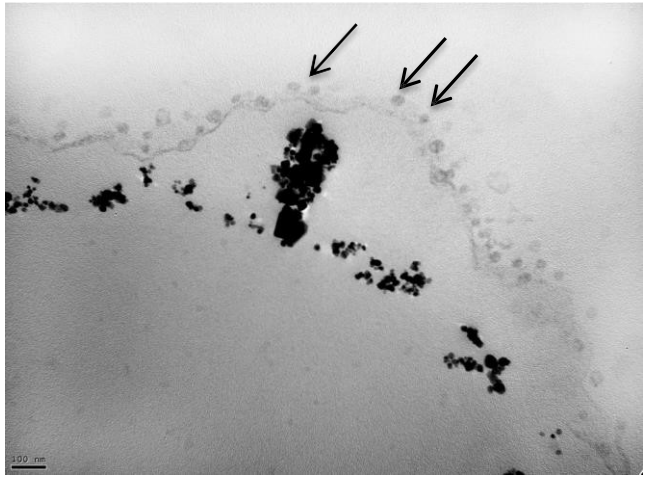
Mass spectrometry

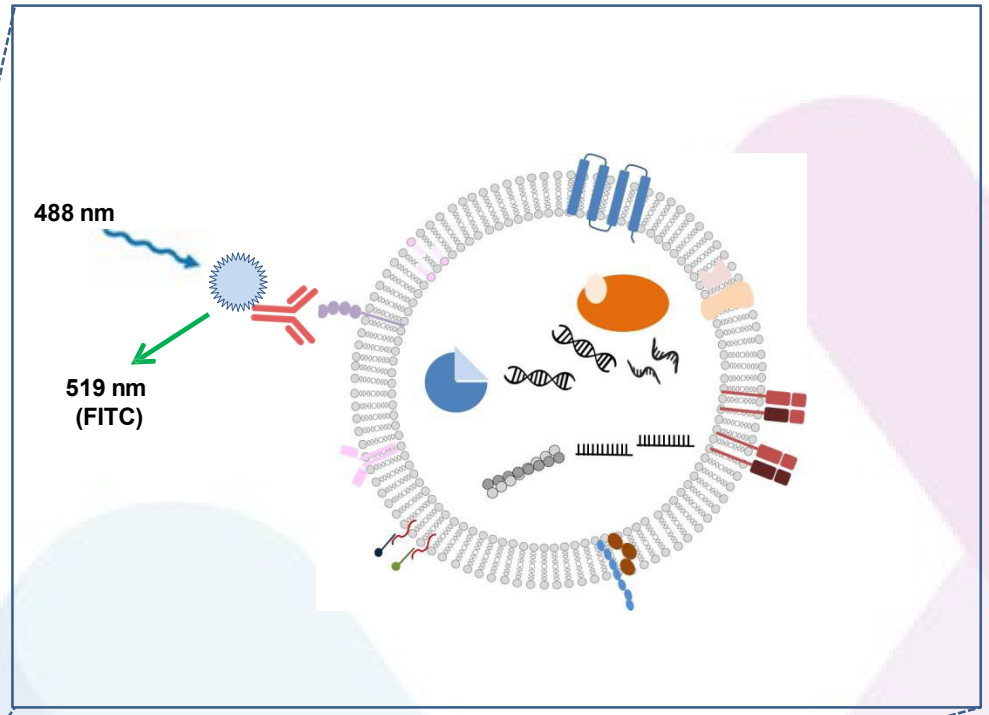


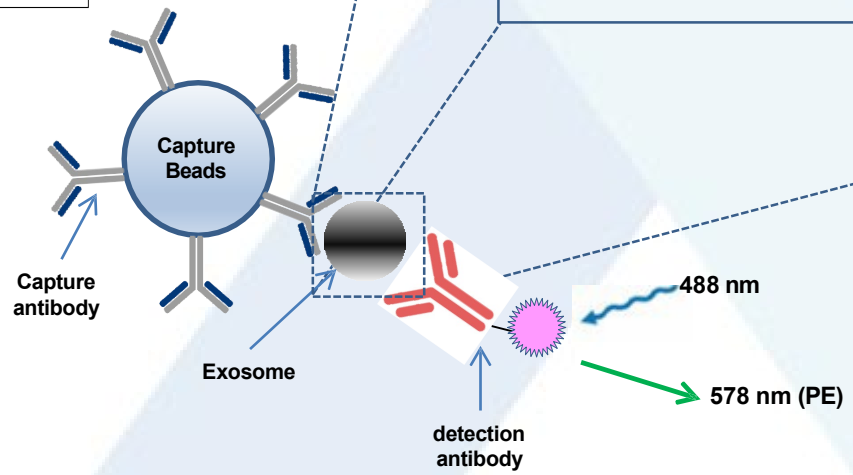
ExoStep: bead-based principle



Electron Microscopy



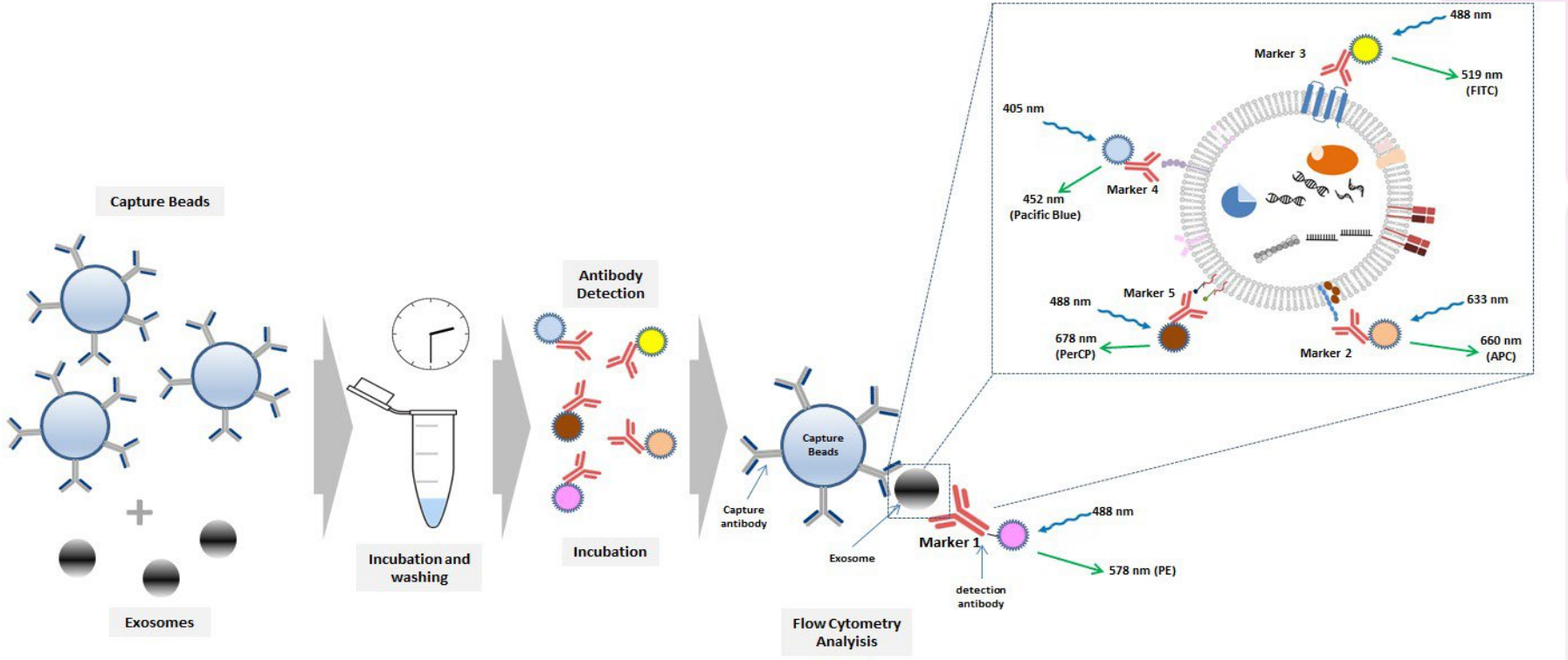




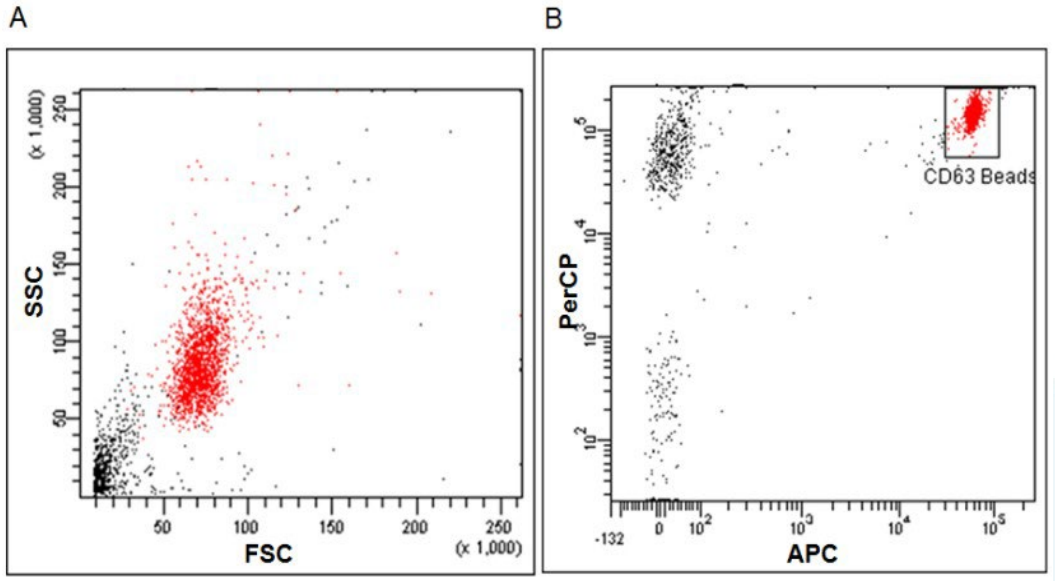
ExoStep Second Generation

Kit components:

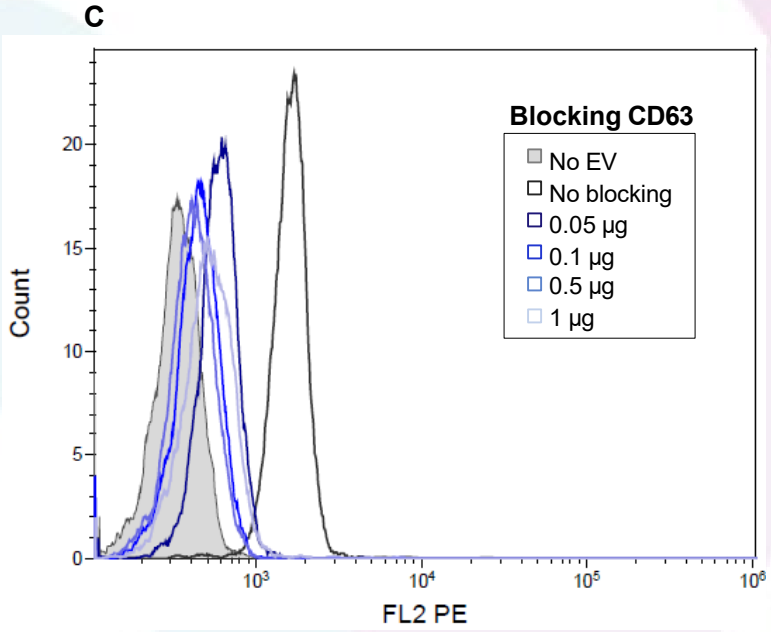
- CD63+ (Clone TEA3/18) capture beads.
- Primary detection antibody, Anti-CD9 PE (Clone VJ1/20) or Anti-CD81 PE (Clone MEM38)
- Assay Buffer 10X, PBS 10% BSA, pH 7,4



Specific and unambiguous exosome detection by Flow Cytometry



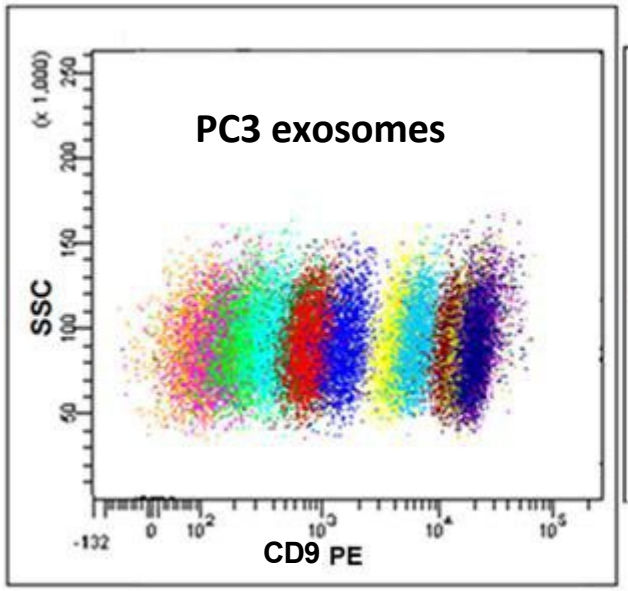
(A) Scatter Dot-plot of beads, FSC vs SSC. (B) Gating strategy on FL3 vs FL4 for flow cytometry acquisition and analysis, in order to remove doublets. Low-speed Flow Cytometry acquisition is recommended

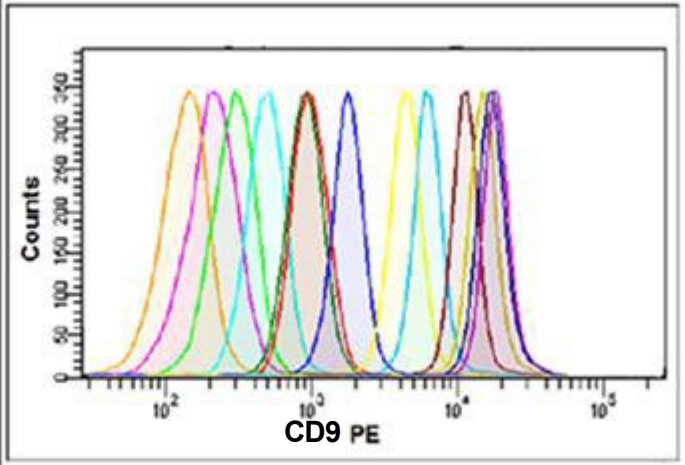


(C) Specificity: antibody blocking. Capture CD63/Detection CD9-biot. 109 particles of PC3-derived EVs were pre-incubated with increasing amounts of the indicated soluble blocking antibody [anti-CD63 (Clone TEA3/18)] before being incubated for capture on CD63- coated beads.

Dynamic range and limit of detection

Greater sensitivity, wide dynamic range. Guaranteed detection even with small sample quantities



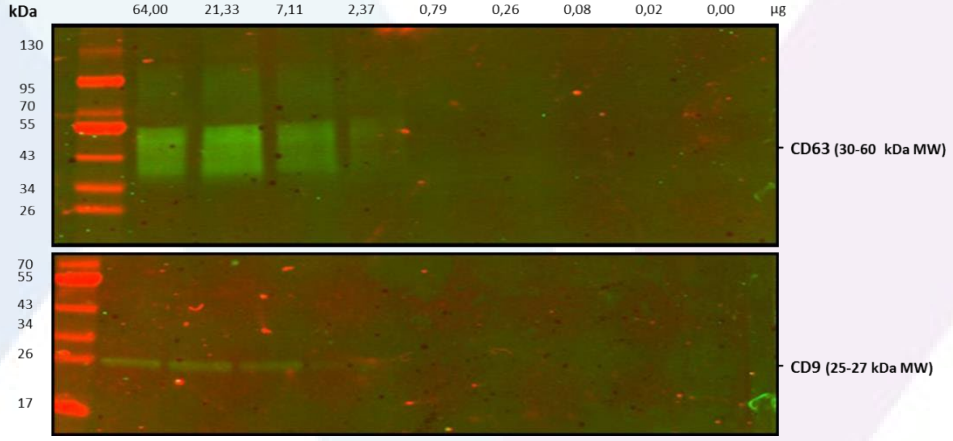


Sensitivity

µg	MFI	SD*
0,0000	8,74	4,06
0,0625	14,20	8,49
0,1250	20,91	14,19
0,2500	36,85	52,61
0,5000	74,32	32,18
1,0000	79,86	82,30
2,0000	158,20	137,14
4,0000	380,00	248,41
8,0000	661,17	257,49
16,0000	1286,40	342,05
32,0000	1746,58	519,01
64,0000	2206,73	537,59
128,0000	2035,14	593,98

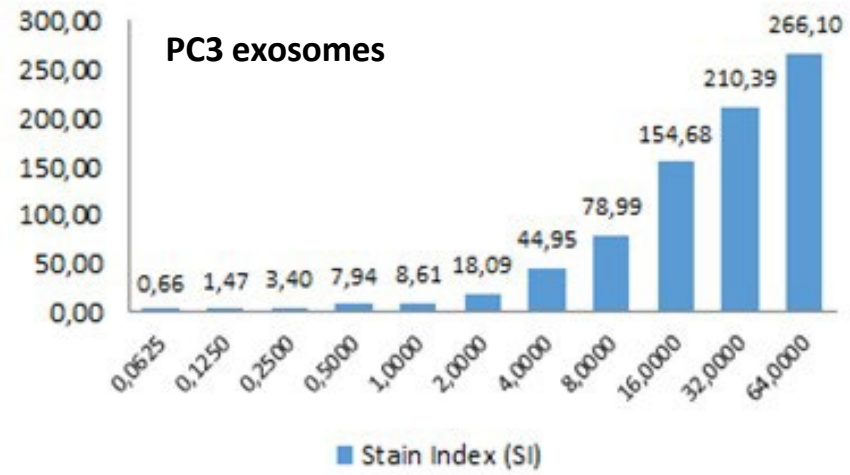
* Standard Desviation.

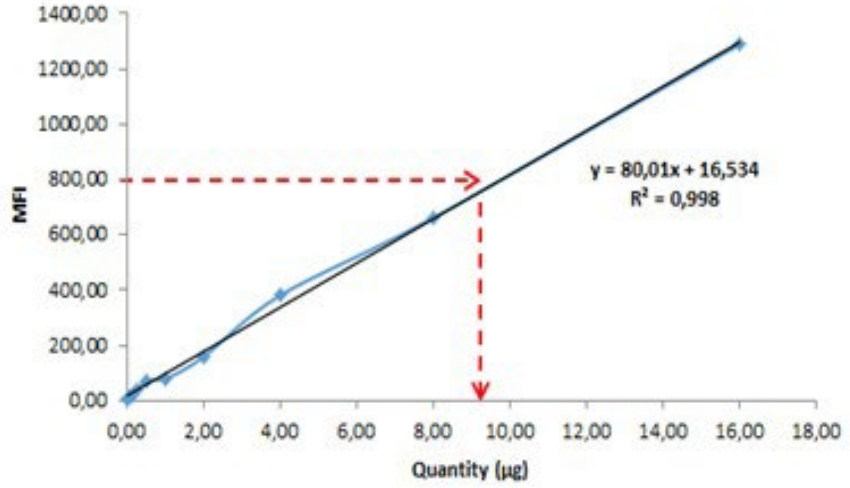
- **LOD: 0,0625 ug -> 7,5 x 10^7 vesicles**
- **LOQ: 0,125 ug - Stain Index (SI) >1**

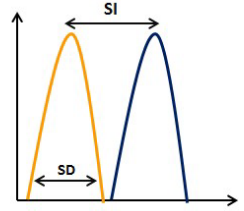


Quantitative analysis

Excellent correlation between fluorescence and the exosome quantity



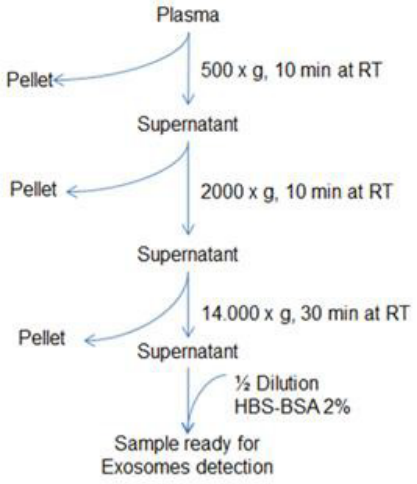


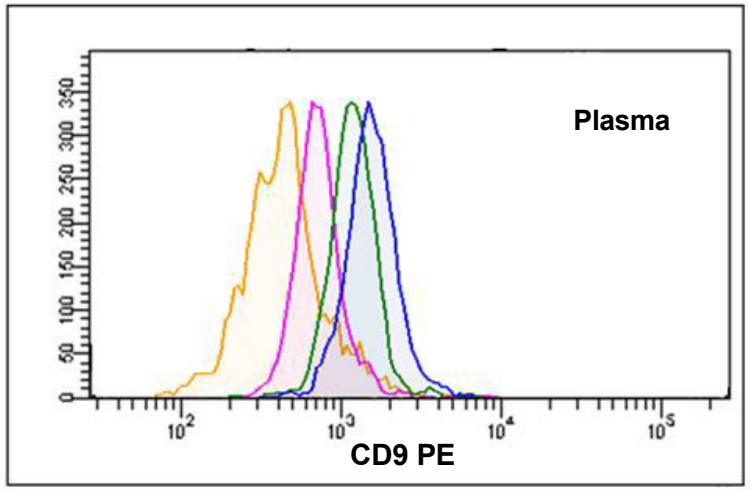


Stain Index = (Median of Positive - Median of Negative) / (SD of Negative * 2)

Direct exosome detection in biological fluids

Isolation no needed

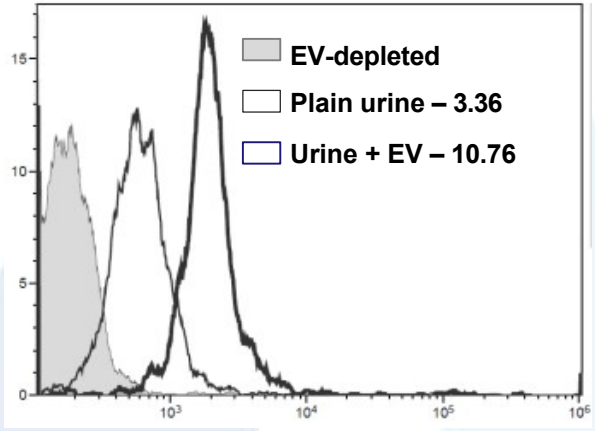


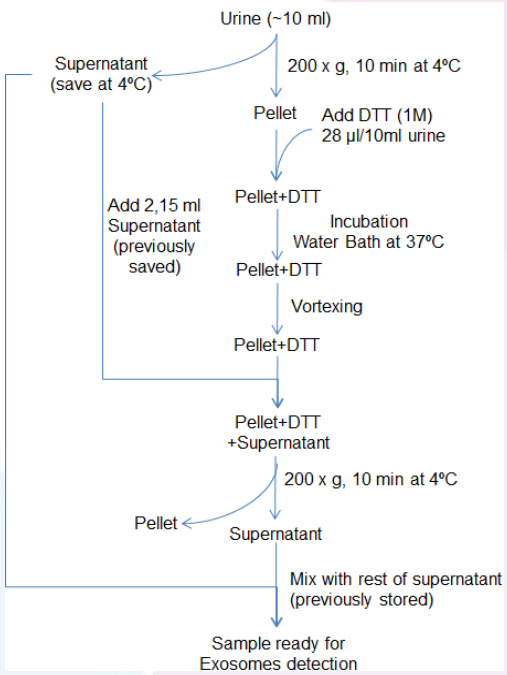


Sensibility for Direct Detection on Plasma (SI))

Vol (ul)	CD63 Beads + CD9 biot + Strep PE
0,00	-
25,00	0,48
50,00	1,52
100,00	2,45

Stain Index (SI) = (Median of Positive - Median of Negative) / (SD of Negative * 2)



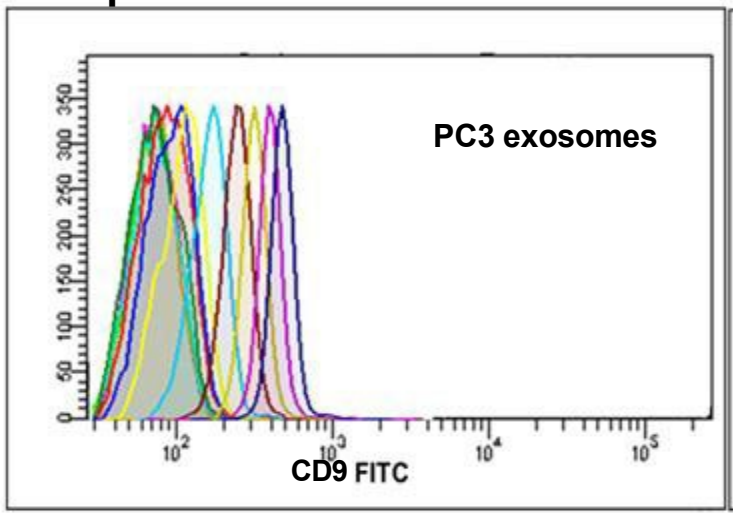


Cell Culture | **Plasma** | **Urine**

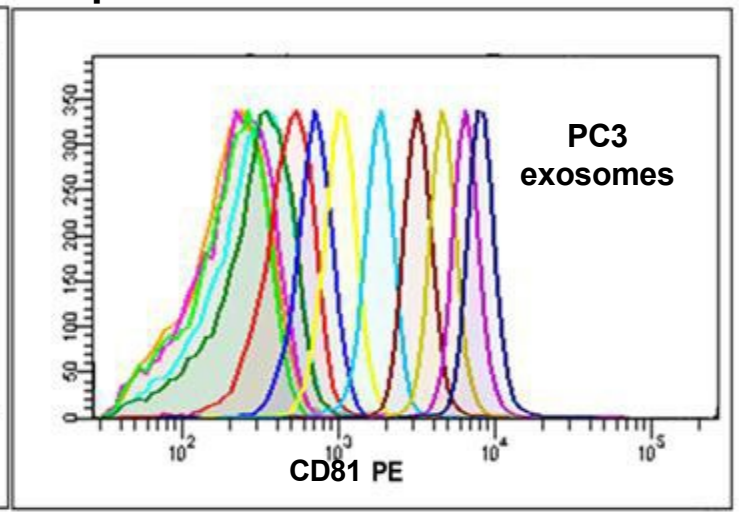
Simultaneous Immunophenotyping

Sensitivity detection in a wide range of quantities

Capture: CD63/ Detection CD9



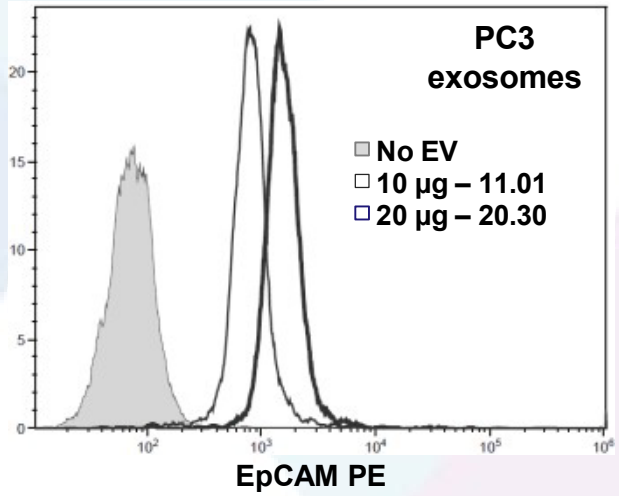
Capture: CD63/ Detection CD81



Immunophenotyping Sensitivity (SI*)		
µg	CD9-FITC	CD81- biot + Strep PE
0,0000	-	-
0,0625	-0,24	0,02
0,1250	-0,24	-0,04
0,2500	-0,30	0,30
0,5000	-0,24	0,45
1,0000	0,12	1,30
2,0000	0,24	2,46
4,0000	0,73	4,39
8,0000	2,06	9,65
16,0000	4,30	20,46
32,0000	6,54	33,62
64,0000	9,56	51,72
128,0000	13,77	69,17

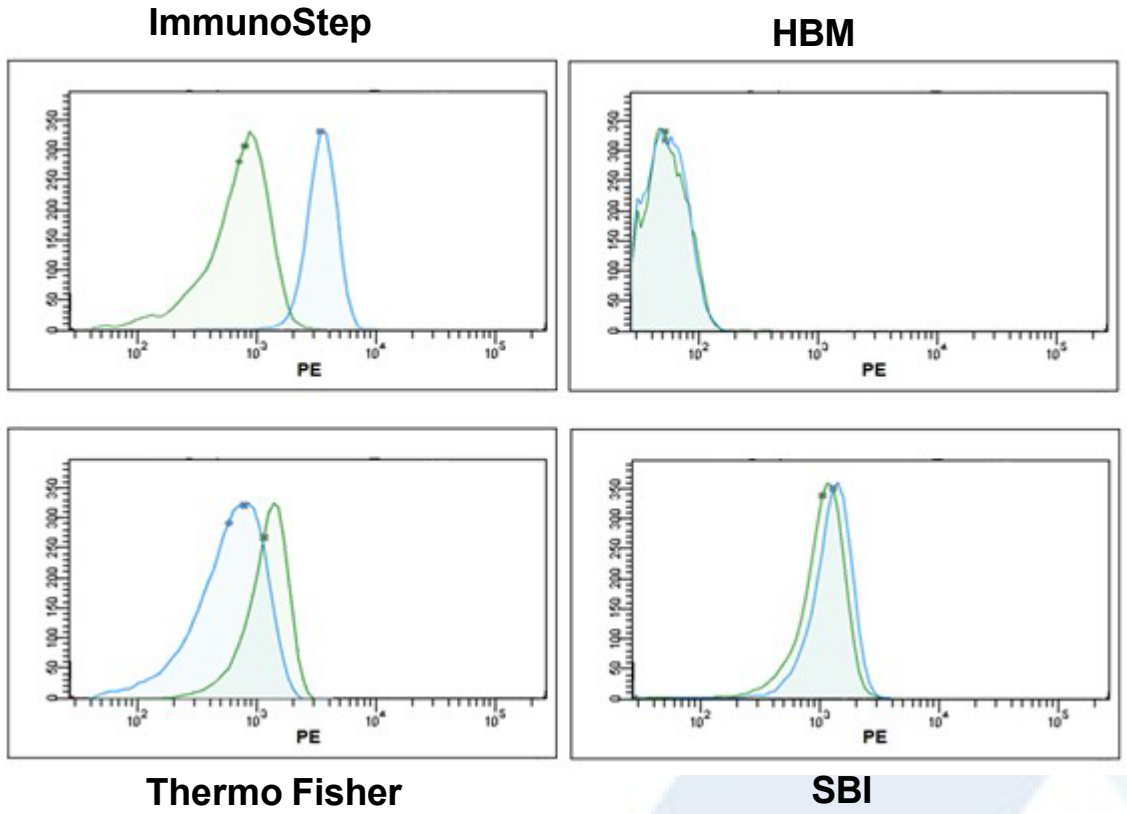
*Stain Index (SI) = (Median of Positive - Median of Negative) / (SD of Negative * 2)

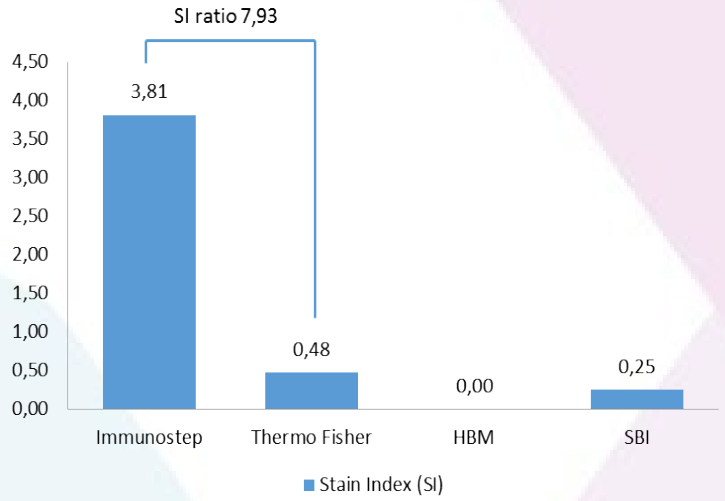
Capture: CD63/ Detection EpCAM



Competence and Competitive Advantage

Sensitivity comparison on same sample





Sensitivity comparison. Data correspond to Stain Index (SI) values obtained by flow cytometry analysis from the 4 different kits on same sample

2µg - 2,40x10⁹ PC3 exosomes

Exosome Analysis Summary

ExoStep kit is a superior alternative for the sensitive detection of exosomes compared to the most commonly used methods, besides being easy to implement and analyze for any laboratory that has access to a conventional flow cytometer

- Specific and unambiguous exosome detection.
- Quantitative analysis, excellent correlationship between MFI and exosomes quantity.
- Direct detection of Exosomes in cell culture supernatant and biological fluids. Without isolation or precipitation.
- Very Small amount of sample needed
- Greater sensitivity, wide dynamic range. Guaranteed detection even with small sample quantities
- Reproducible
- Allowing simultaneous immunophenotyping of exosomes capture population

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EXCELENCIA SEVERO OCHOA

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MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD

fUAM 25 años 1991-2016 Fundación de la Universidad Autónoma de Madrid

immunostep we know how

Thanks you for your attention