
we know how
immunostep
our vision
Experts in helping to
improve diagnosis by
flow cytometry

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immunostep

IMMUNOSTEP is a Spanish biotechnology company that develops, produces and markets reagents and diagnostic devices, for use in flow cytometry.

A team of experts in flow cytometry and development of antibodies for diagnosis

- Spin Off of the University of Salamanca (Cytometry Service) – Acknowledged with multiple awards.
- More than 50 products and DIV> 850 references in its catalog.
- Technological portfolio with several patents and licenses.
- Among its customers, hospitals, research centers and important companies.

IMMUNOSTEP QUALITY

- ✓Manufacturer Health License number: 5463-PS
- ✓Immunostep is an ISO 9001:2008 Certified company*
- ✓ Immunostep is an ISO 13485:2013 Certified company. Medical Devices certification*

LICENCIA SANITARIA PREVIA DE FUNCIONAMIENTO
DE INSTALACIÓN DE PRODUCTOS SANITARIOS

DENOMINACIÓN DE LA EMPRESA	NÚMERO DE LICENCIA
INMUNOSTEP, S.L.	5463 P.S.

CIF/NIF: B 37373784
DOMICILIO: AVDA. UNIVERSIDAD DE COIMBRA S/N. CAMPUS MIGUEL DE UNAMUNO 37007 SALAMANCA
ACTIVIDAD PROPIA: FABRICACIÓN DE PRODUCTOS SANITARIOS PARA DIAGNÓSTICO "IN VITRO"
ACTIVIDADES CONCERTADAS:
TIPO DE PRODUCTO: ANTICUERPOS MONOCLONALES FRENTE A ANTÍGENOS LEUCOCITARIOS HUMANOS PUROS, CONJUGADOS, KIT DE DIAGNÓSTICO Y SOLUCIÓN DE FIJACIÓN Y PERMEABILIZACIÓN PARA CITOMETRIA DE FLUJO, TUBOS DE RECOLECCIÓN CON SOLUCIÓN ESTABILIZANTE
TÉCNICO RESPONSABLE: D. ALFREDO CONDE ALONSO
TITULACIÓN: LICENCIADO EN BIOLOGÍA

MADRID, 09 DE DICIEMBRE DE 2015

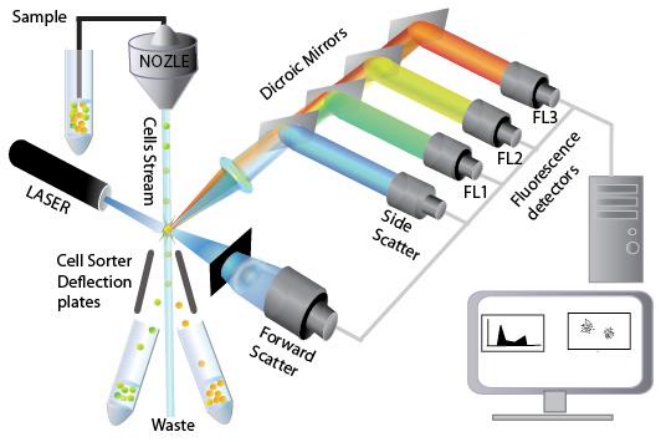
LA DIRECTORA DE LA AGENCIA ESPAÑOLA DE MEDICAMENTOS Y PRODUCTOS SANITARIOS

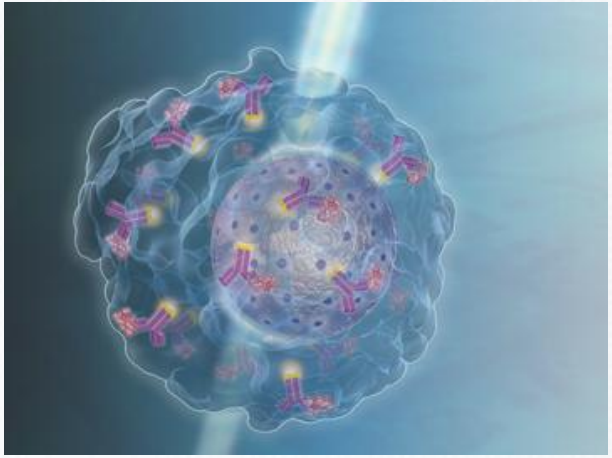
MANUFACTURER HEALTH LICENSE

ISO9001:2008

ISO13485:2013

ABOUT FLOW CYTOMETRY

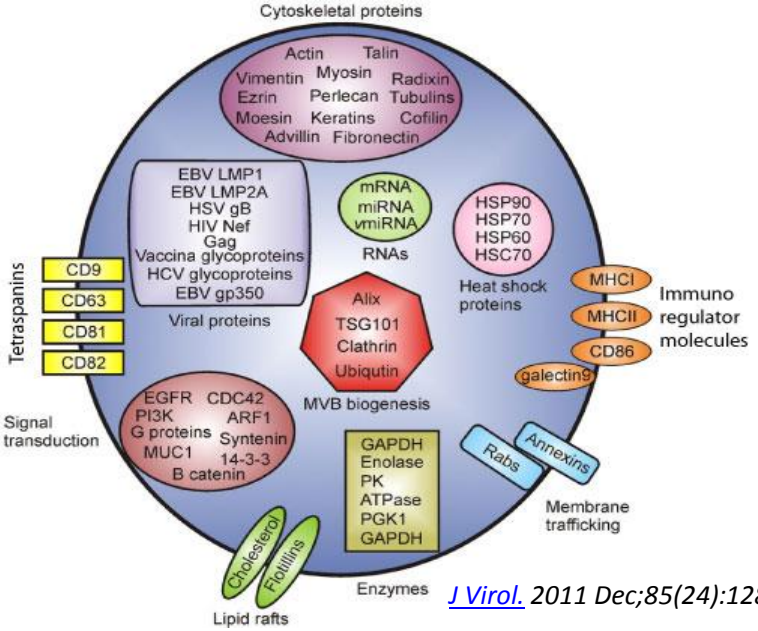




Flow cytometry is a technology based on the use of laser light, employed in the counting, cell sorting, biomarker detection by suspending cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and chemical characteristics of up to thousands of particles per second.

EXSOMES BACKGROUND

Exosomes are small extracellular vesicles that are released from cells upon fusion of an intermediate endocytic compartment, the multivesicular body (MVB), with the plasma membrane. They are thought to provide a means of intercellular communication 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



J Virol. 2011 Dec;85(24):12844-54

Exosomes are present in many bilogical fluids:

- **Serum/plasma**
- **Urine**
- **Cerebrospinal fluid**
- **Saliva**
- **Amniotic fluid**

MECHANISM & FUNCTIONS

Key mechanism:

- Direct contact between surface molecuels of exosomes and cells
- Endocytosis of exosomes
- Exosome-cell membrane fusion
- Horizontal transfer of oncogenic receptors
- Transfer of HIV vesicles

Key funcions:

- Antigen presentation
- Immunostimulatory and inhibitory activities.

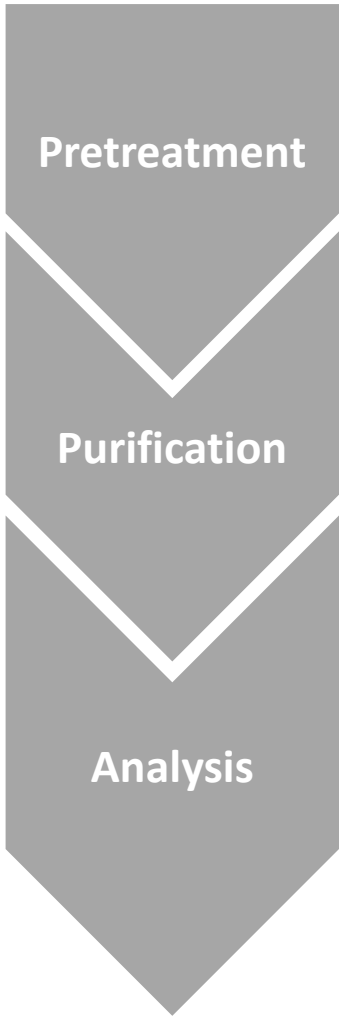
Importance:

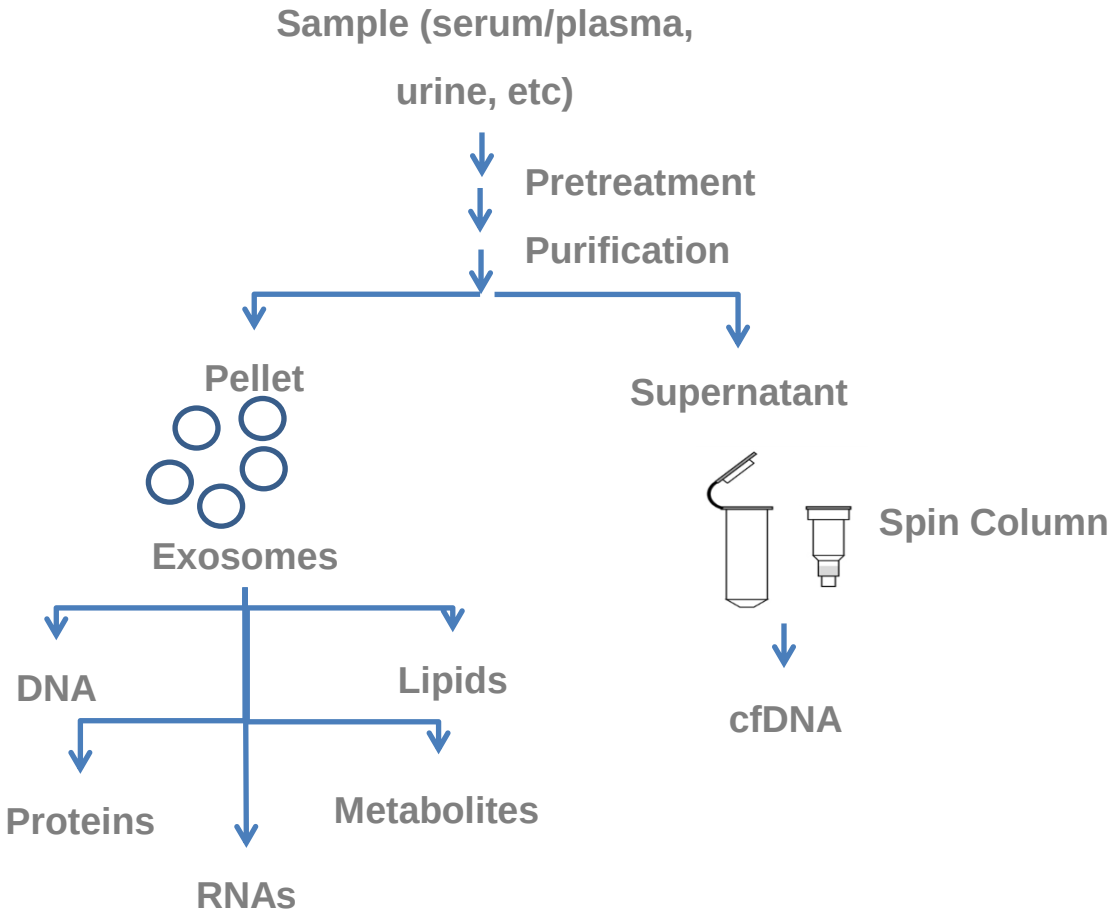
- Provide a means of intercellular communication and of transmission of macromolecules between cells
- Role in the spread of proteins, lipids, mRNA, miRNA and DNA and as contributing factors in the development of several diseases
- Proposed to be useful vectors for drug delivery.

KEY FEATURES

Key features of Exosomes:	
Size range	Approximately 40-200 nm
Mechanism of generation	By exocytosis of MVBs
Isolation	Differential centrifugation and sucrose gradiente ultracentrifugation 100.000 – 200.000 b, vesicle density is 1,13 – 1,19 g/ml
Detection	TEM, WB, mass spectrometry, flow cytometry (bead coupled)
Best characterized cellular sources	Immune cells (DCs, T cell, B cells, macrophages) and tumors
Markers	Annexin V binding, CD63, CD9,CD81, CD9, TGS101

EXOSOMES WORKFLOW





Exosomes are cell-screted nanovisicles (40-200 nm) that represent a rich source of novel biomarkers in the diagnosis and prognosis of certain disease.

Relevance as biomarkers

Isolation & analysis

Limited detection

Heterogeneous composition

Technical Challenge

Development a novel flow cytometry immunoassay for the isolation and detection of exosomes based on the use of tetraspanins as targets

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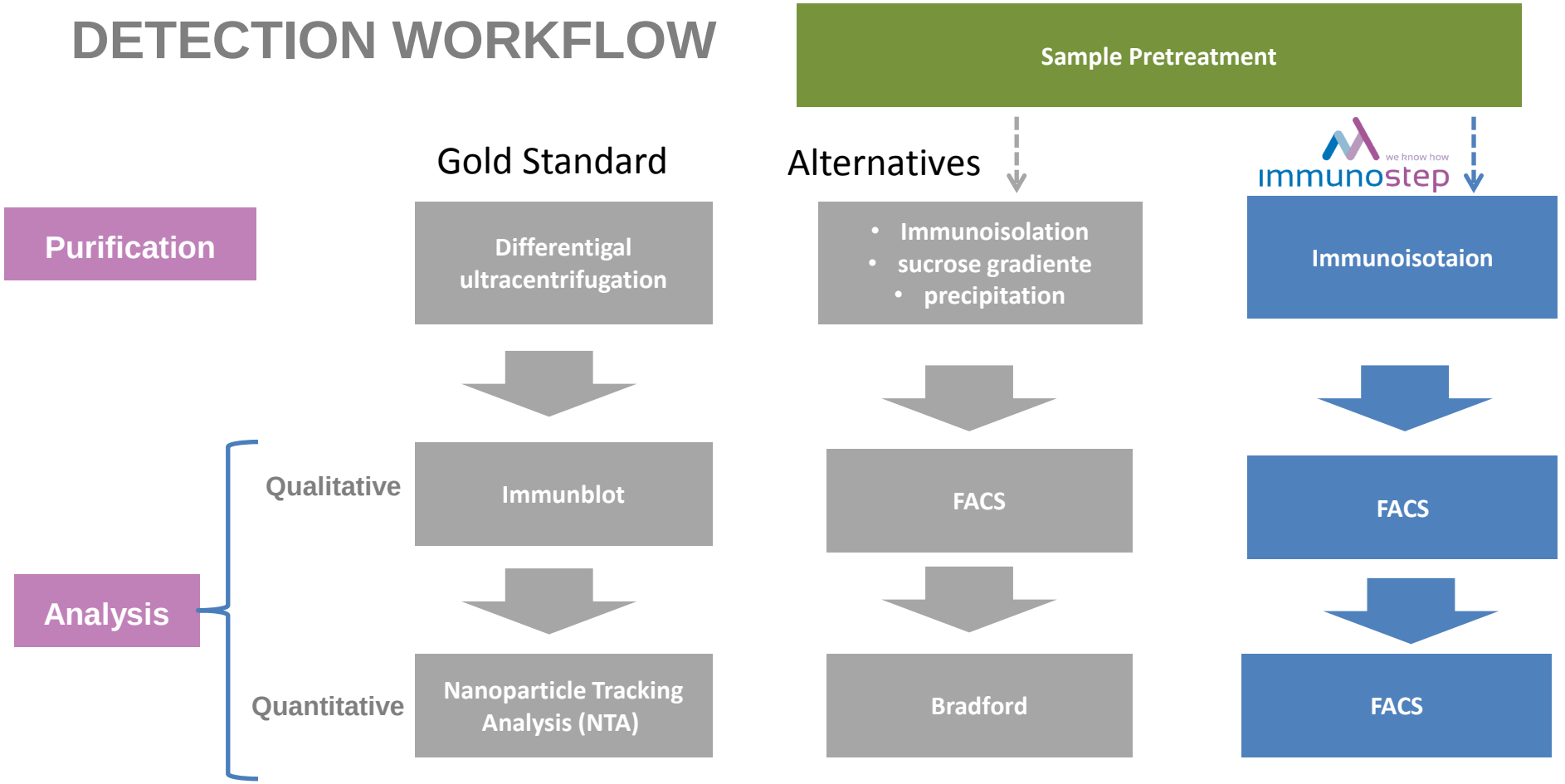
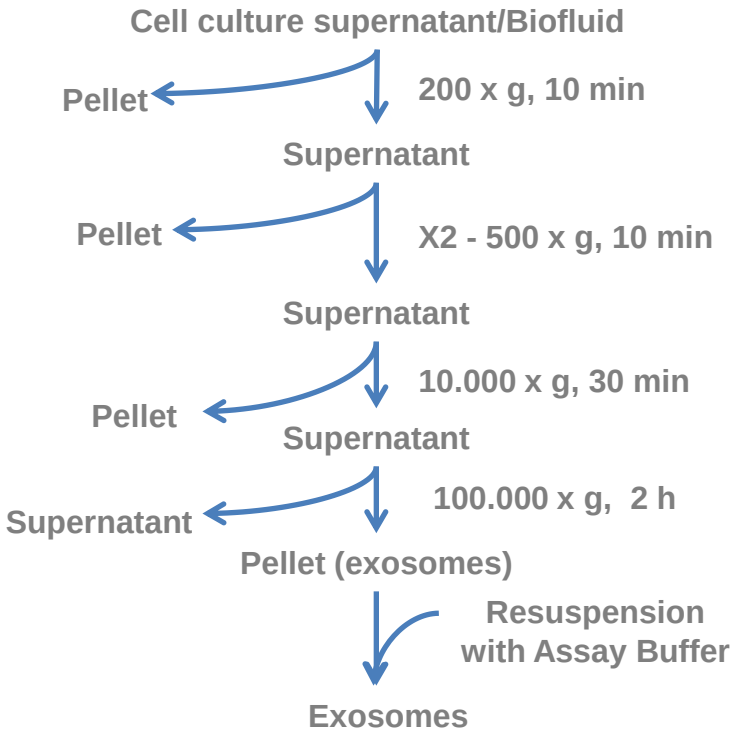
EXOSOME ISOLATION AND DETECTION WORKFLOW



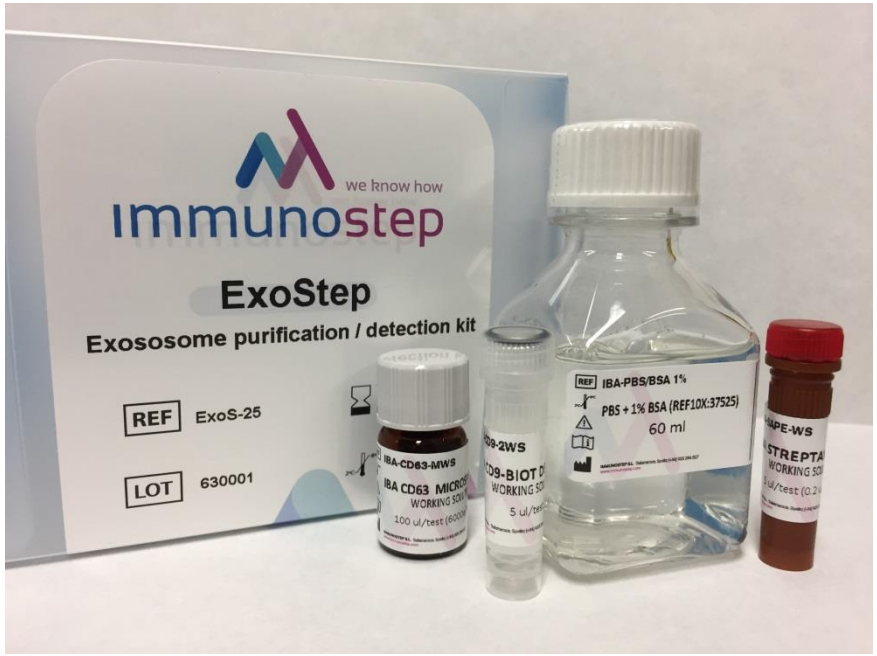
PURIFICATION OF EXOSOMES BY DIFFERENTIAL ULTRACENTRIFUGATION

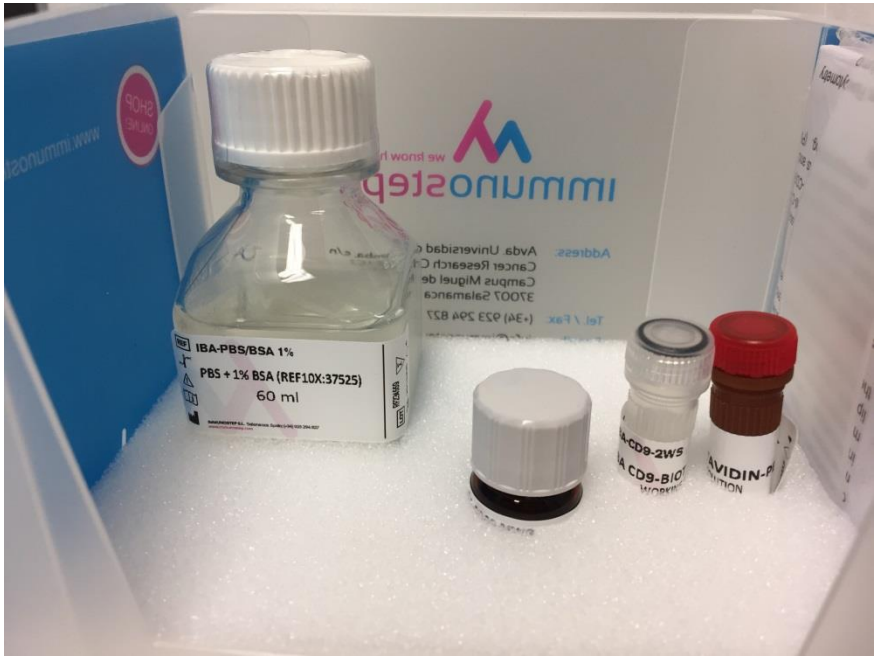


EXOSTEP: EXOSOME PURIFICATION AND FACS ANALYSIS KITS

PRODUCT	DESCRIPTION	FLUORO	REF	SIZE	REAGENTS PROVIDED
ExoStep Cell Culture	ExoStep™ Culture Exosome purification and FACS analysis kit of exosomes derived from cell culture	Phycoerythrin (PE)	ExoS-25-C	25 test	• CD63+ (Clone TEA3/18) capture beads. • Anti-CD9 biotin (Clone VJ1/20) • Streptavidin-Phycoerythrin (PE) • Assay Buffer 10X
ExoStep Plasma	ExoStep Plasma Exosome purification and FACS analysis kit of exosomes derived from human plasma	Phycoerythrin (PE)	ExoS-25-P	25 test	• CD63+ (Clone TEA3/18) capture beads. • Anti-CD9 biotin (Clone VJ1/20) • Streptavidin-Phycoerythrin (PE) • Assay Buffer 10X • HBS-BSA 2%
ExoStep Urine	ExoStepTM Unine Exosome purification and FACS analysis kit of exosomes derived from human urine	Phycoerythrin (PE)	ExoS-25-U	25 test	• CD63+ (Clone TEA3/18) capture beads • Anti-CD9 biotin (Clone VJ1/20) • Streptavidin-Phycoerythrin (PE) • Assay Buffer 10X • Dithiothreitol buffer 1 M

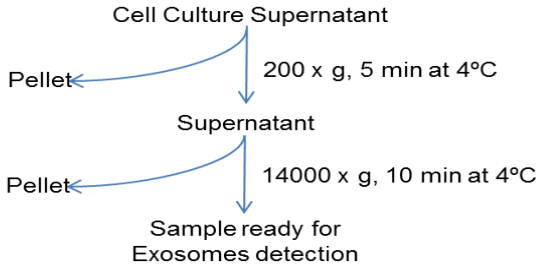
EXOSTEP PRESENTATION

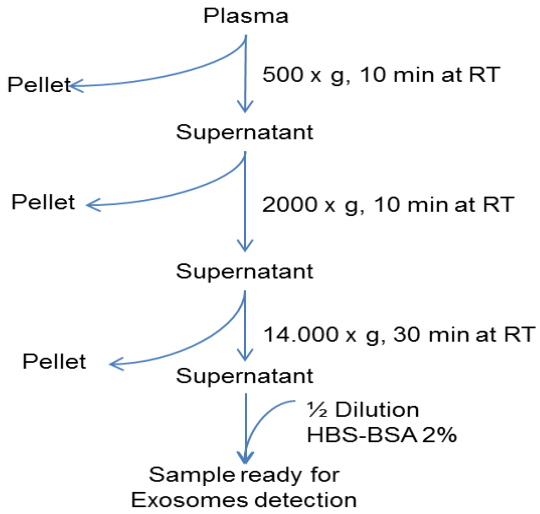


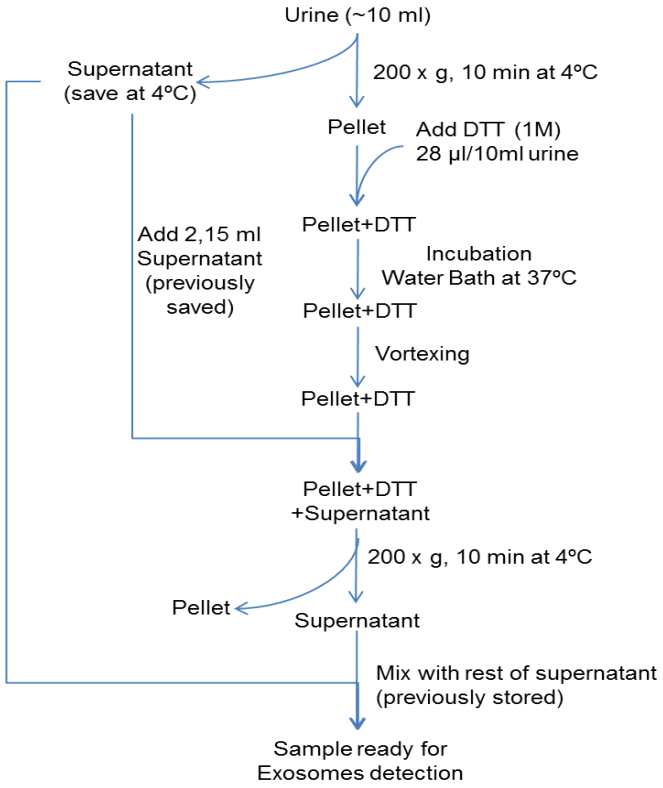


SAMPLE PRETREATMENT FOR DIRECT EXOSOME DETECTION ON SAMPLE

Ultracentrifugation







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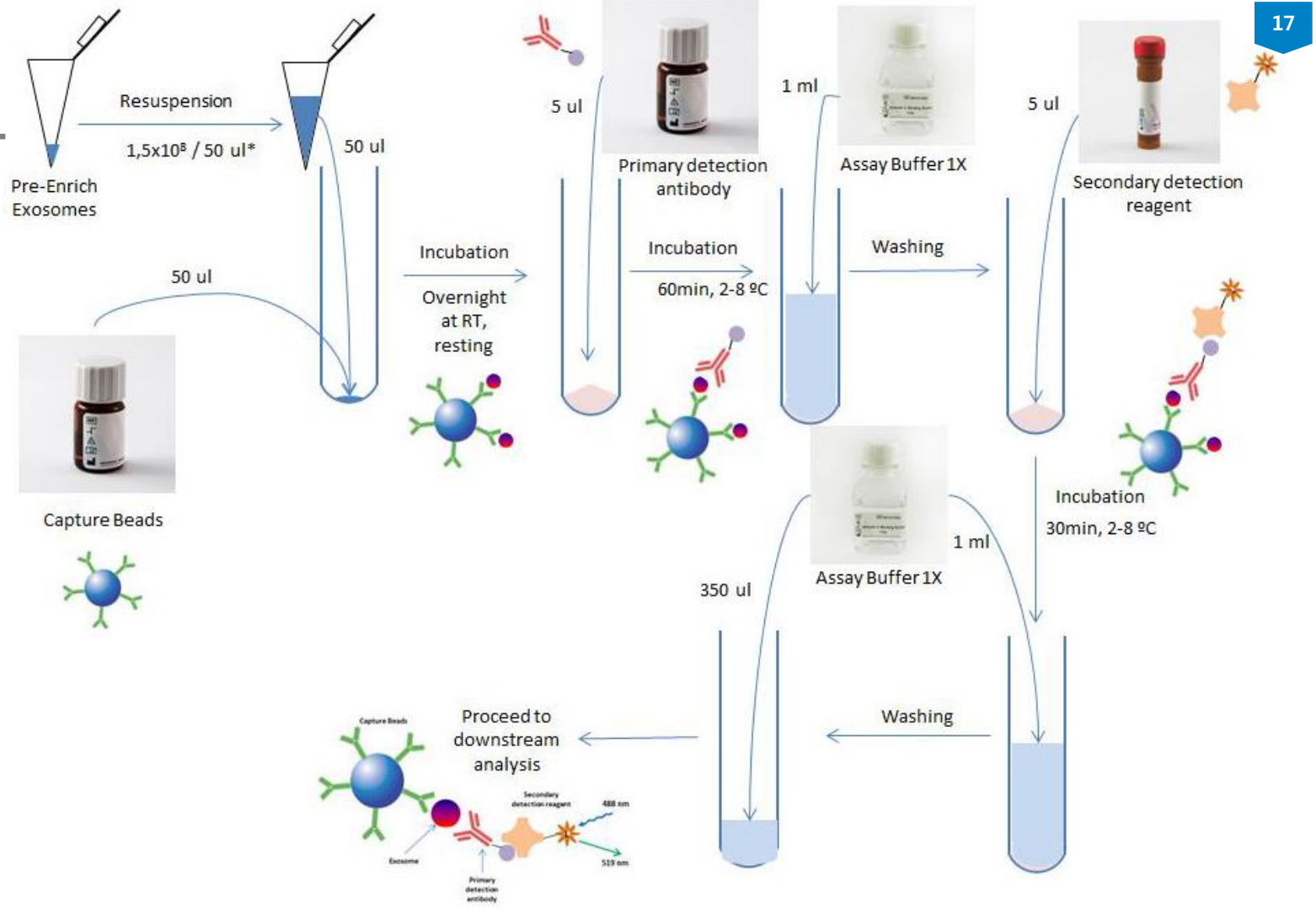
EXOSTEP PROTOCOL



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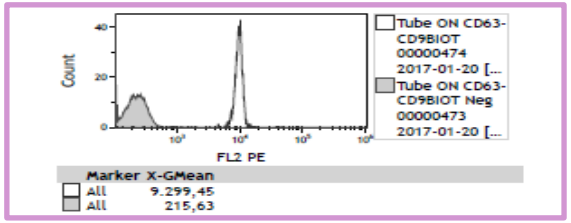
COMPARISON ON AND 2 HOURS

Comparison tube, U-plate, ON: aCD63 bead- aCD9BIOT detector

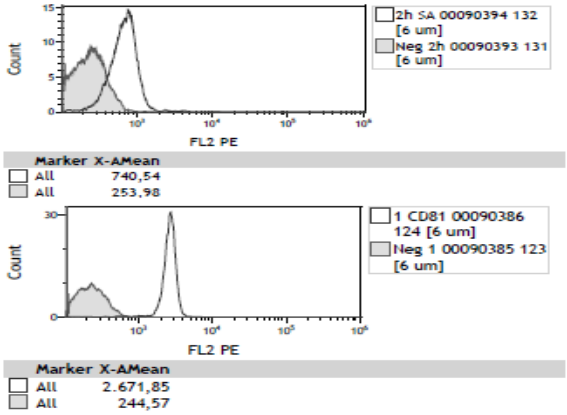


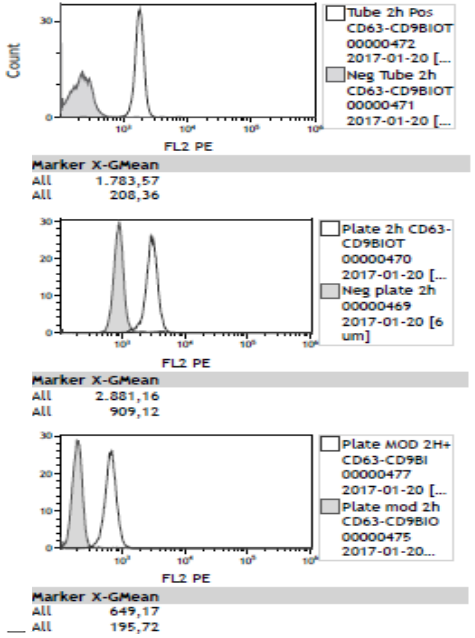
Best option

If you want faster results…

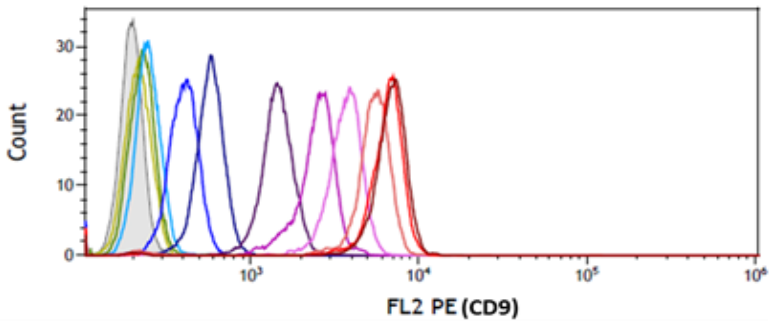
You will loose resolution but, you can still detect your ptotein

aCD81-BIOT with 6000 CD9 beads and 2 ug PC3 exosomes, 3 ul SA





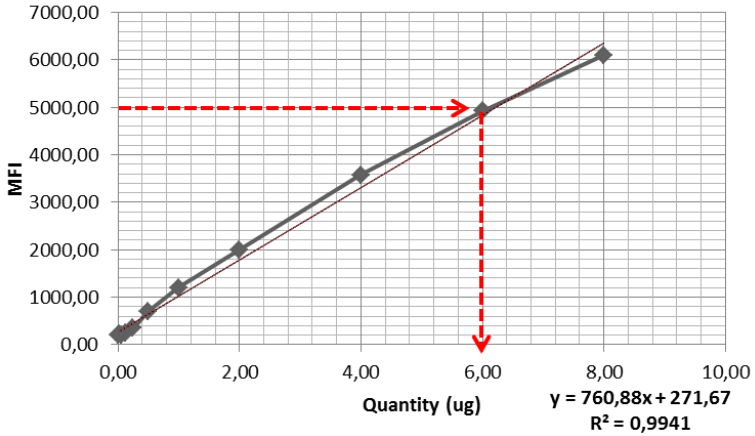
DYNAMIC RANGE



Quantity (ug)	MFI
10,00	6387,02
8,00	6098,75
6,00	4920,21
4,00	3572,29
2,00	2326,23
1,00	1401,62
0,50	564,62
0,25	397,72
0,13	246,74
0,06	226,41
0,03	214,58
0,00	198,12

With 8-10 µg of PC3 exosomes we are very near to the saturation of the curve.

Limit of detection (LOD): 0,125 ug



CONCENTRATION ESTIMATION FROM FLOW CYTOMETRY EXOSOME DATA

PC3 exosmes LOD could be set a 0,125 ug, ($12x10^8$ exosomes/ul) correspond to $1,5x10^8$ particles.

EXOSOME RELATED PRODUCTS

PRODUCT	DESCRIPTION	FLUOROCROME	REF	SIZE
CD63+ capture beads.	CD63+ (Clone TEA3/18) capture beads for human exosome isolation.	N/A	63Bead-25	25 test
Streptavidin	Streptavidin is useful for detecting biotinylated antibodies.	PE	STV-50	50 µg
anti-human CD63 Antibody	CD63+, clone TEA3/18, is a monoclonal antibody intended for human exosome detection using flow cytometry in combination with ExoStep kits	Biotin, CFBLUE, FITC PE	63XExo-50	50 µg
anti-human CD81 Antibody	CD81, clone M38, is a monoclonal antibody intended for human exosome detection using flow cytometry in combination with ExoStep Kits	Biotin, CFBLUE, FITC, PE	81XExo-50	50 µg
anti-human CD9 Antibody	CD9, clone VJ1/20, is a monoclonal antibody intended for human exosome detection using flow cytometry in combination with CD63+ (Clone TEA3/18) capture beads.	Biotin, CFBLUE, FITC, PE	9XExo-50	50 µg
anti-human CD59 Antibody	CD59, clone VJ1/12,2, is a monoclonal antibody intended for the exosome detection using flow cytometry in combination with ExoStep Kit.	Biotin, CFBLUE, FITC ,PE	59XExo-50	50 µg
anti-human EpCam (CD326) Antibody	CD326 (EpCAM), clone VU-1D9, is a monoclonal antibody intended for the exosome detection using flow cytometry in combination with ExoStep Kit.	Biotin, FITC ,PE	326XExo-50	50 µg
Biotin anti-human NKG2D (CD314) Antibody	CD314 (NKG2D), clone 1D11, is a monoclonal antibody intended for the exosome detection using flow cytometry in combination with ExoStep Kit.	Biotin, FITC ,PE	314XExo-50	50 µg

EXOSTEP: ADVANTAGES

- Biological fluids validated (serum, plasma, urine)
- Extensive Cell line Validation (HEK, MCF7, HT-29, PC3, SkMel, SUM 159, T24, etc)
- Sample Exosomes Direct Detection.
- Reproducible results and can be run in parallel to exosome immunophenotyping.
- Best alternative to WB, higher sensitivity and dynamic range.
- Specific detection and Relative quantification of exosomes, avoiding contamination with other proteins.
- Autoflorescence and magnetic beads for easy identification and handling.

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- Exclusive technology worldwide
- Broad Product & Services Porfolio
- Reliable partner

Let's talk

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