

Driving Research, Use and Engineering of Exosomes

騰達行產品專員
李佳玟

Outline

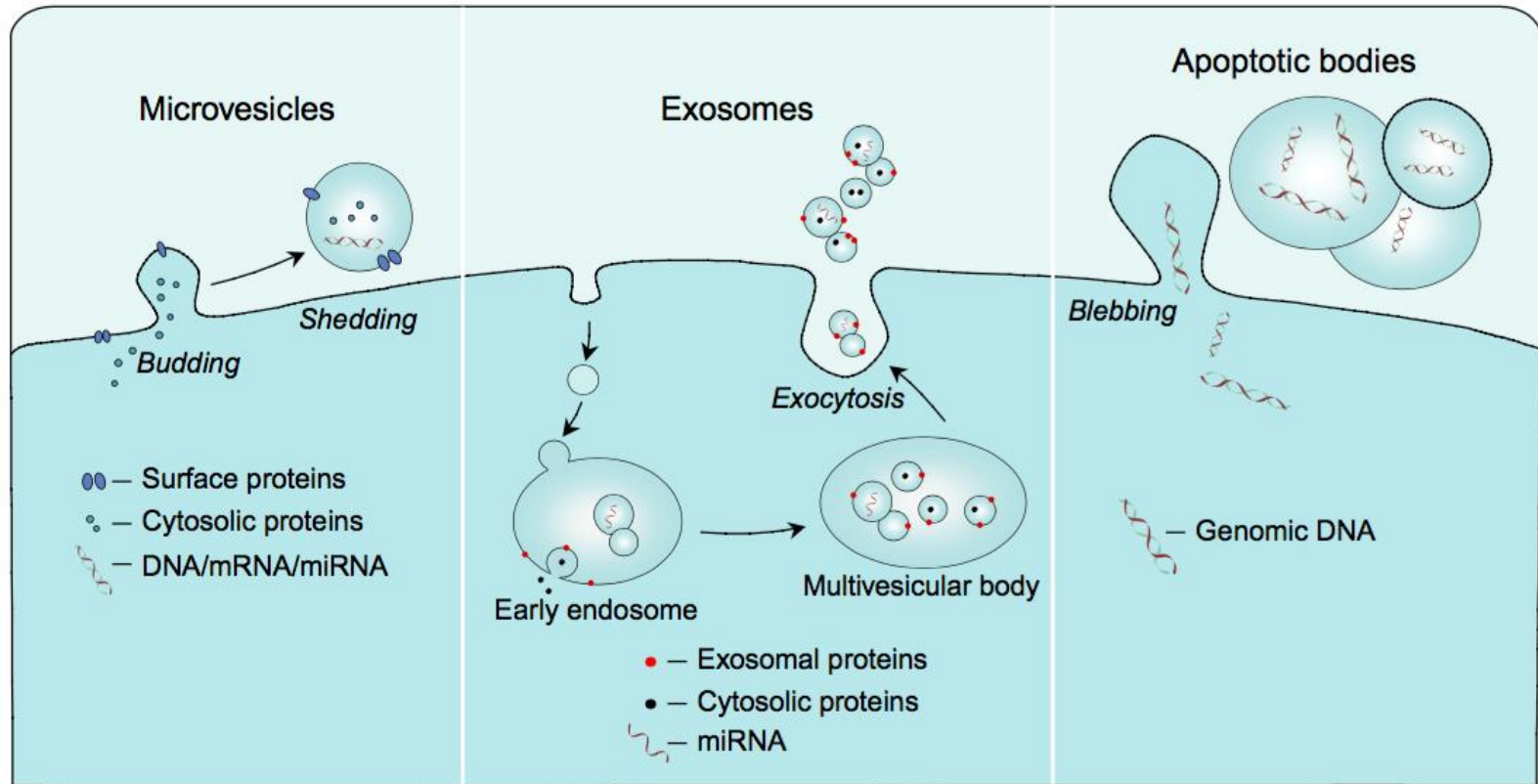
- **What are Exosomes?**
- **Function and Application of Exosomes**
- **Research tools for study of Exosomes**
 - Isolation
 - Purification
 - Detection
 - Quantitation
 - Discovery
 - Engineering & Delivery



Lonza

HansaBioMed Life Sciences – Now Part of Lonza

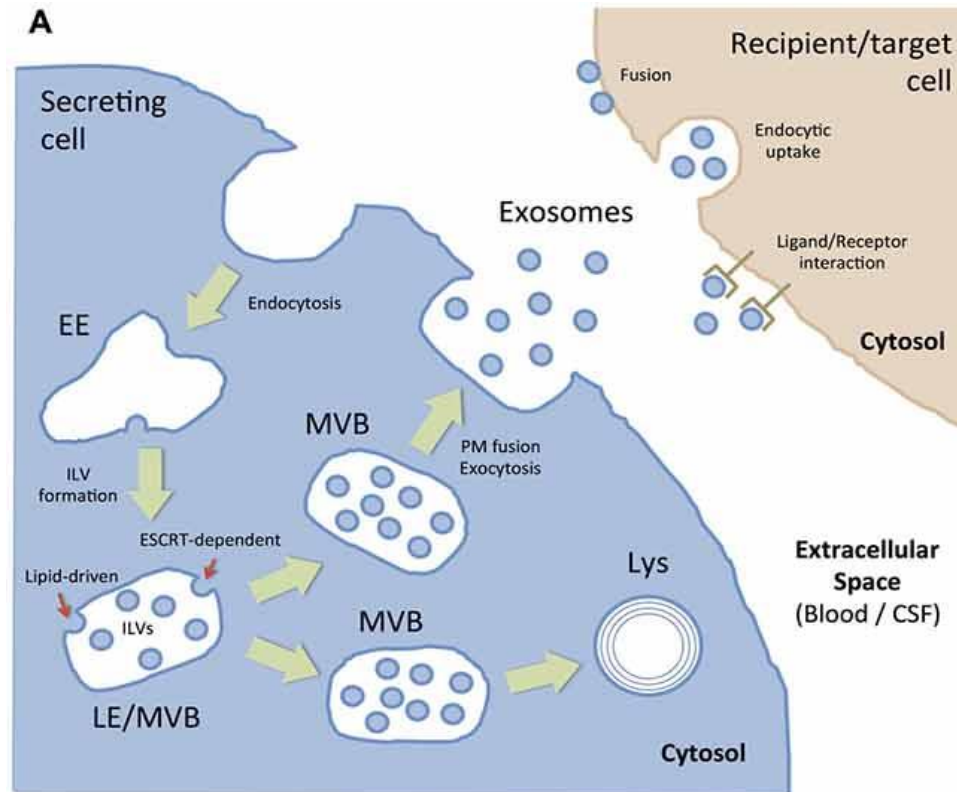
Extracellular Vesicles (EVs)



- Size: 100-1000 nm
- Markers: integrins, selectins & cell specific markers
- Size: 30-100 nm
- Markers: Rab5, CD63, CD9, CD81
- Size: 1-5 μ m

Exosome Features

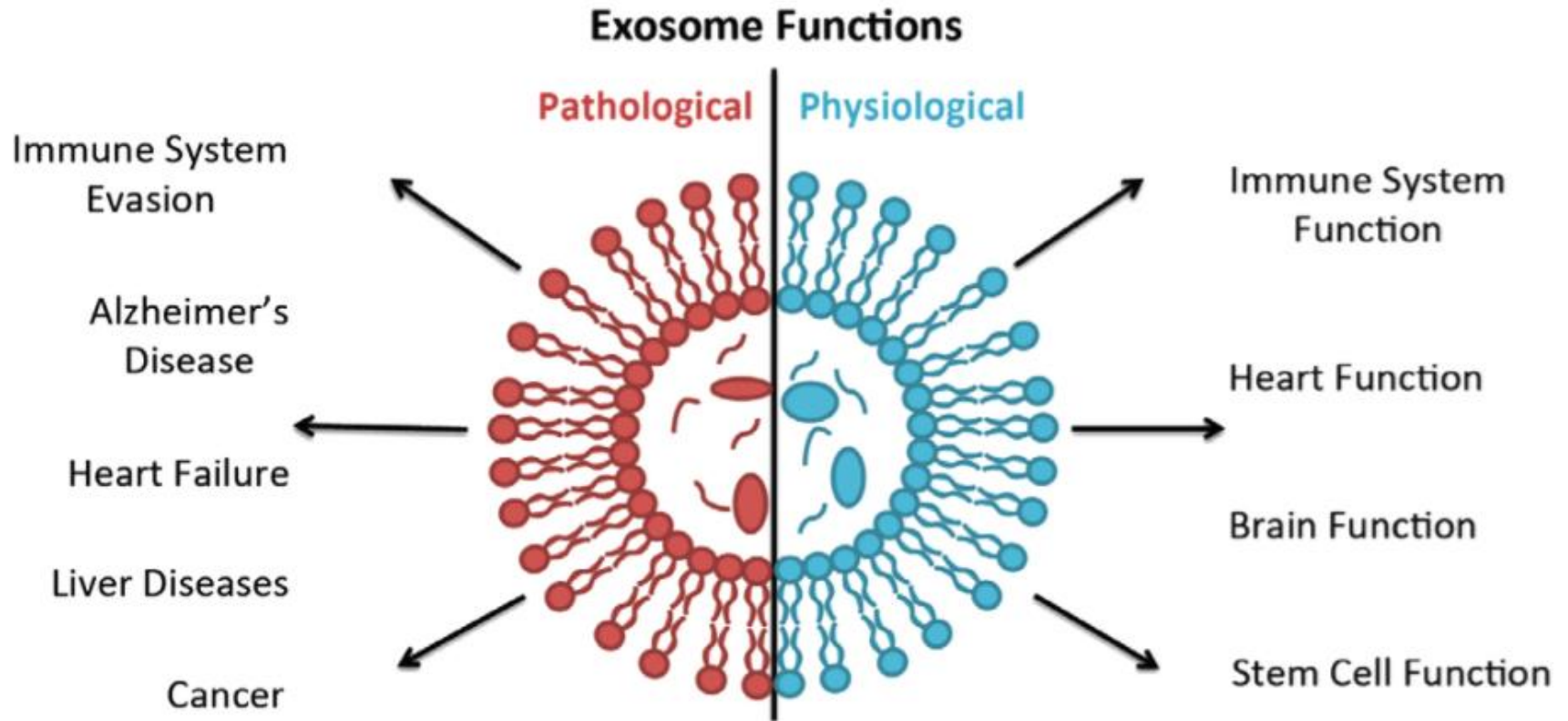
- Size: 30-100nm vesicles
- Secreted by a wide range of cells
- Composition depends on cell of origin
- Biomolecules : Proteins: membrane bound & free, enzymes, RNAs: coding RNAs & ncRNAs



Why are exosome Important?

- **Mediate intercellular communication** in both physiological and pathological conditions (cancer, inflammation, immunity, CNS function, cardiac cell function)
- Discover exosome-associated **biomarkers** and develop targeted **diagnostics**
- **Potential therapeutic vehicles** in disease for targeted drug delivery

Exosomes & other EVs in Cell-to-Cell Communication



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Biomarkers and targeted diagnostics

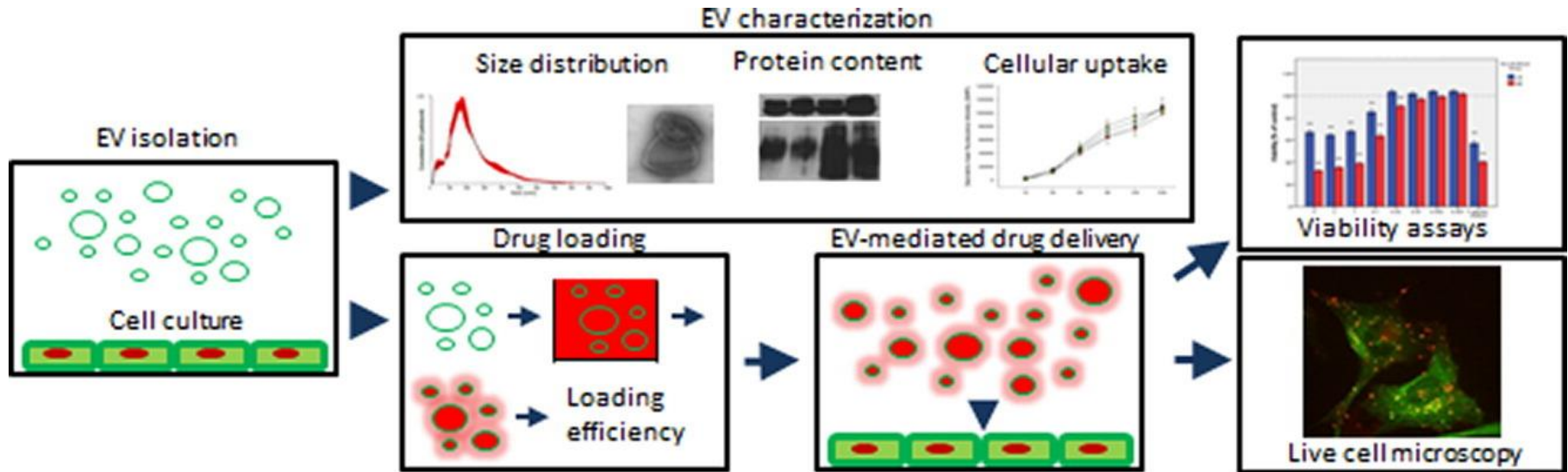
Cargo	Cancer type	Patient cohorts	Exosome source (isolation method)	Assay used	Outcome and/or utility
DNA					
KRAS ^{G12V} , KRAS ^{G12D} , KRAS ^{G12R} , KRAS ^{G12C} , KRAS ^{G12S} , KRAS ^{G12A} , and KRAS ^{G13D} DNA	PDAC	39 patients with PDAC and 82 healthy controls	Plasma (ultracentrifugation)	ddPCR	Exosomal mutant KRAS DNA was better for predicting PDAC status than cell-free mutant KRAS DNA ²⁴⁰
RNA					
AR-V7 mRNA	CRPC	26 patients receiving abiraterone and 10 patients receiving enzalutamide	Plasma (exoRNeasy kit)	ddPCR	AR-V7 mRNA predicted resistance to hormonal therapy; overall survival was significantly shorter in exosomal AR-V7-mRNA ⁺ participants than in exosomal AR-V7-mRNA ⁻ participants (3 months versus 20 months) ²⁴¹
Protein					
GPC1	PDAC	190 patients with PDAC and 100 healthy controls	Serum (ultracentrifugation)	Fluorescence-activated cell sorting	GPC1-positive exosomes distinguished healthy individuals and patients with benign pancreatic disease from patients with early-stage and late-stage PDAC with 100% specificity and 100% sensitivity ¹⁶²

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Exosomes as drug delivery agent

- Load **chemotherapeutic agents, siRNAs, and miRNAs**

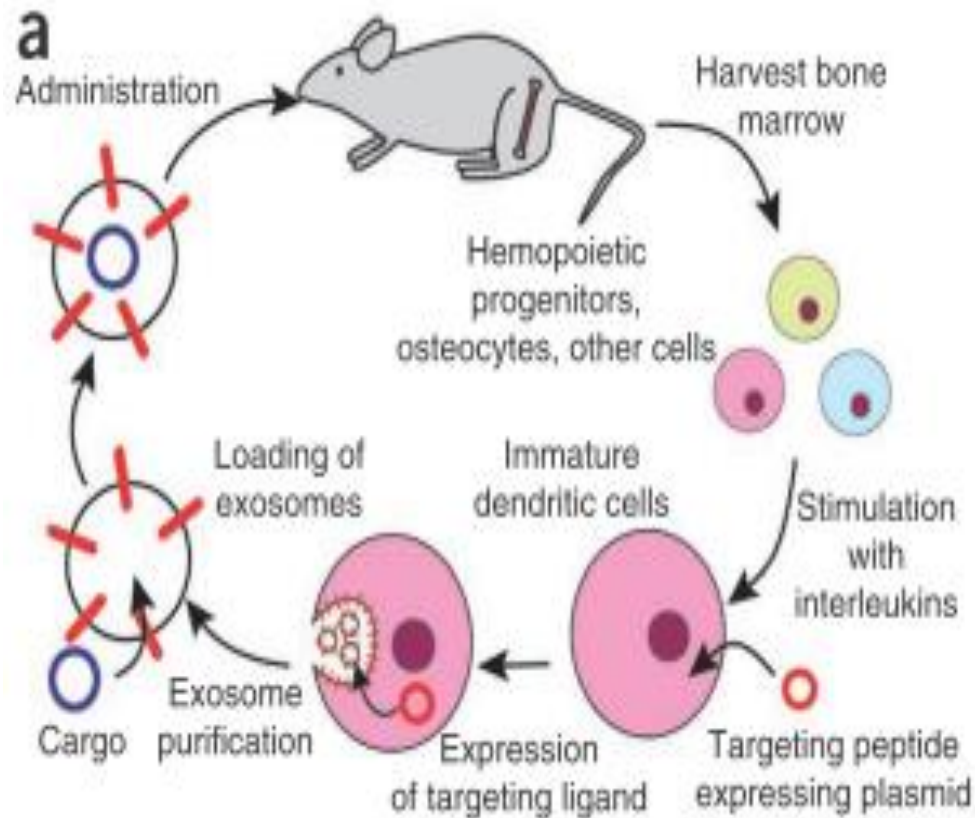


LNCaP and
PC3 prostate
cancer cells

- PtX-loaded EVs were prepared by incubating 1×10^8 – 5×10^9 EVs/mL in 1 mL of 5 μ M PtX-DPBS solution for 1 h at 22 °C.
- Trypsin-treated PtX-EVs, 0.25% trypsin was added to the samples and incubated at 37 °C for 30 min.

Exosomes as drug delivery agent

- Package active proteins, nucleic acid, and other active molecules into exosomes, where cells are transfected with an **engineered effector-expressing vector**.



Exosome Research Tools

Exosome Isolation

- [ExoQuick-ULTRA EV Isolation Kit](#)
- [Exo-FBS](#)
- [ExoQuick-TC EV Isolation Kit](#)

[View Products](#)

Exosome Detection

- [ExoCheck Exosome Antibody Array](#)
- [ExoFlow-ONE EV Labeling Kit \(Emerald\)](#)
- [ExoFlow-ONE EV Labeling Kit \(Garnet\)](#)

[View Products](#)

Exosome Quantitation

- [ExoELISA-Ultra Complete Kit \(for CD63 Detection\)](#)
- [ExoELISA-Ultra Complete Kit \(for CD81 Detection\)](#)
- [FluoroCet Exosome Quantitation Kit](#)

[View Products](#)

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Exosome Isolation



General: total EVs

- Ultracentrifugation
- Polymer-based (ExoQuick family)
- Filtration (membrane-based or SEC columns)

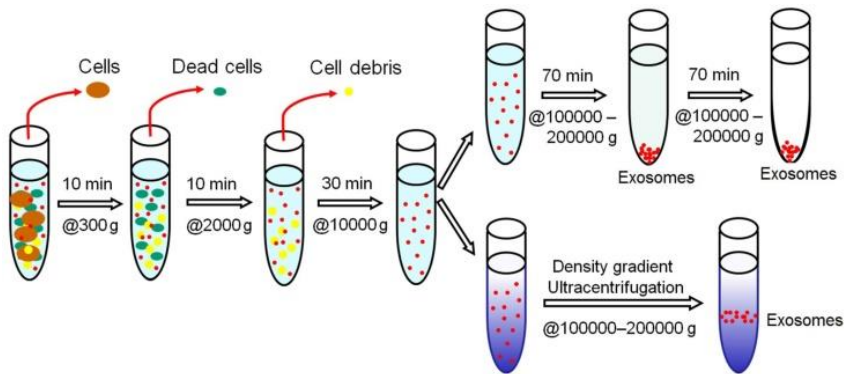
Marker-specific: subpopulation

- Affinity-based

General Isolation Methods

Traditional method

Ultracentrifugation



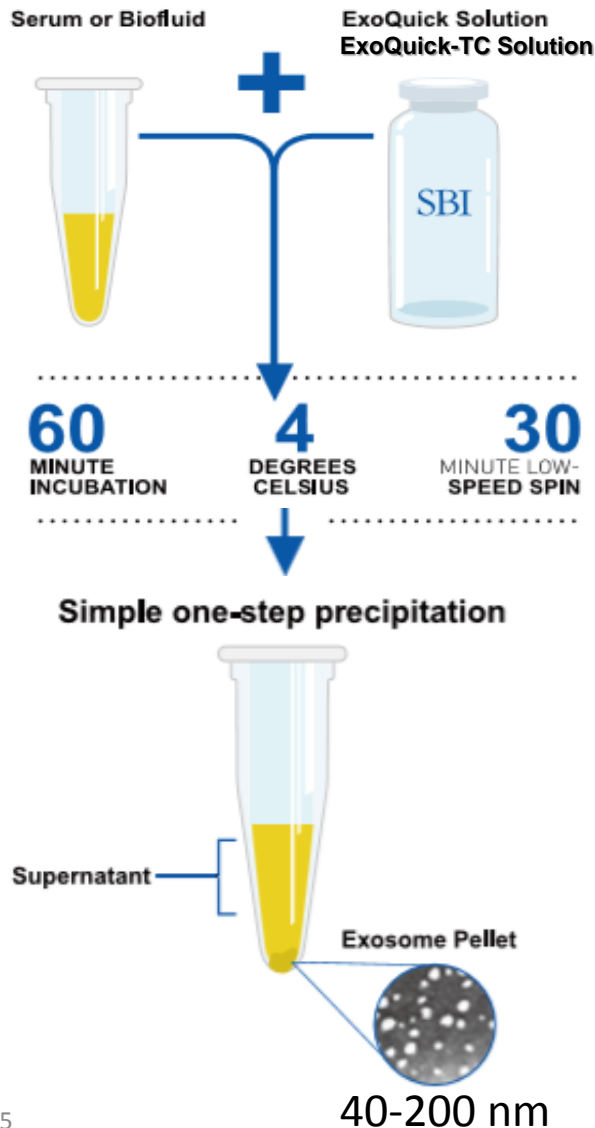
SBI's polymer-based method

ExoQuick Family

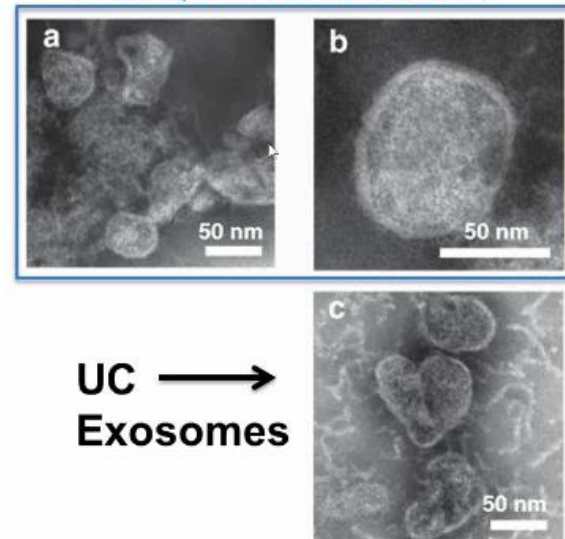
Product	Biofluid
Original ExoQuick	Serum, plasma & ascites
ExoQuick-TC	All other biofluids & cell culture medium
Exo-Quick-LP	Removes lipoproteins from plasma/serum
ExoQuick Plasma prep	De-fibrinates plasma before exosome isolation
ExoQuick-CG	Preparing exosomes used in pre-clinical <i>in vivo</i> applications
ExoQuick-TC ULTRA	Sensitive applications, e.g. mass spec, exosome labelling, <i>in vivo/ex vivo</i> delivery

How to Use ExoQuick & ExoQuick-TC?

- No time-consuming ultracentrifugation
- Efficient with low sample volumes (as little as 100 μ l)



ExoQuick Exosomes



UC \longrightarrow
Exosomes

T Umezu, et al. *Leukemia cell to endothelial cell communication via exosomal miRNAs.* *Oncogene* (2013) 32, 2747–2755 July 2012

ExoQuick/ ExoQuick TC ULTRA

1. Add **ExoQuick ULTRA** to your sample and incubate

2. Spin 3,000g x 10 min

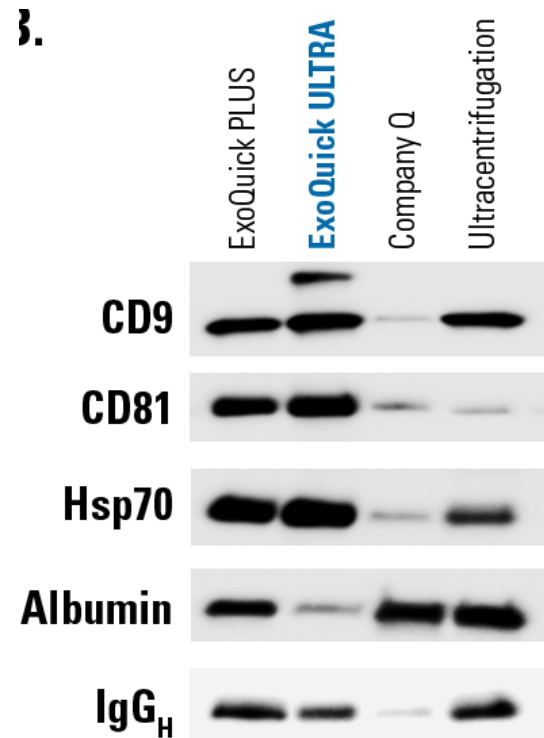
Pellet
Supernatant

3. Resuspend EVs and add to pre-washed **ExoQuick ULTRA** columns

4. Spin 1,000g x 30 sec and collect EVs



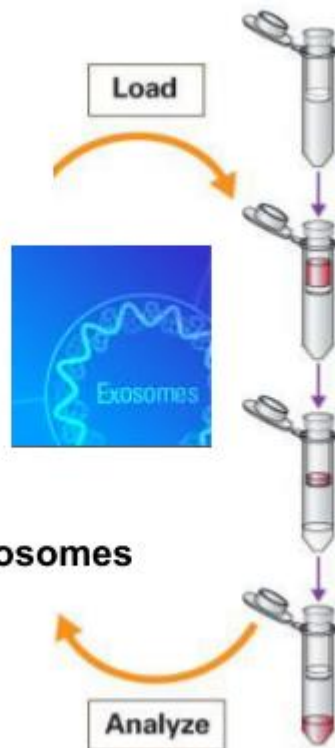
- Reduces protein carryover
- Increases purity for downstream protein detection applications (mass spectrometry, western blotting)



Capturem Exosome Isolation Kit (Cell Culture)

Collect supernatant from cultured cells → Run through pre-clearing column → Run through isolation column → Wash → Elute

25 ml
Cell Culture
Media
(pre-clear)

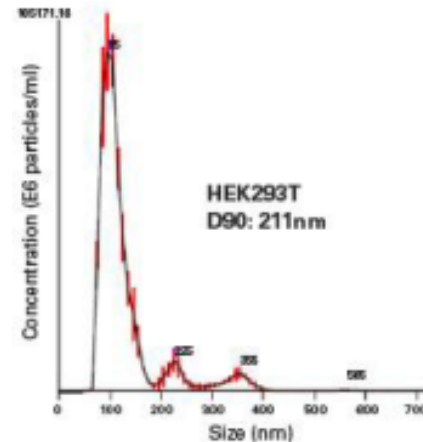
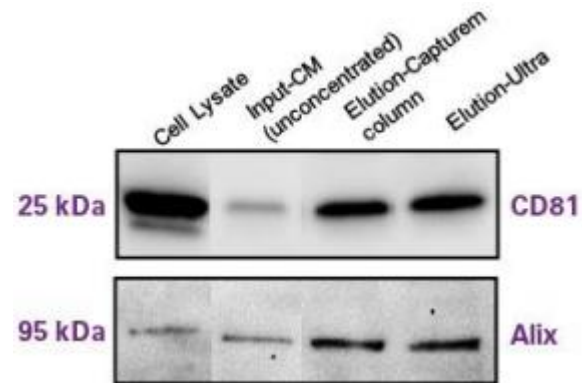


Bind

Wash

Elute

$\sim 2 \times 10^{11}$ exosomes



Exosome Isolation



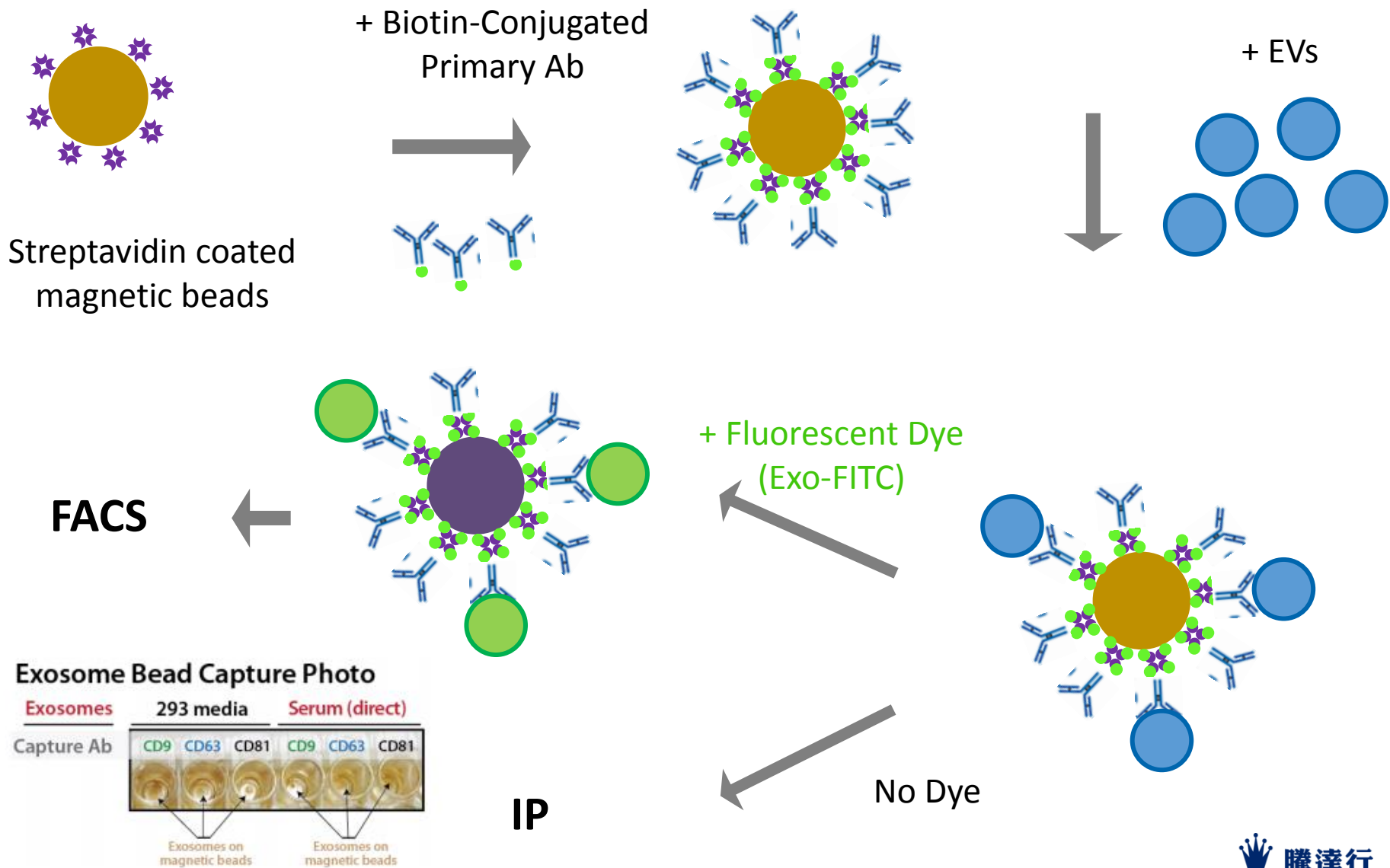
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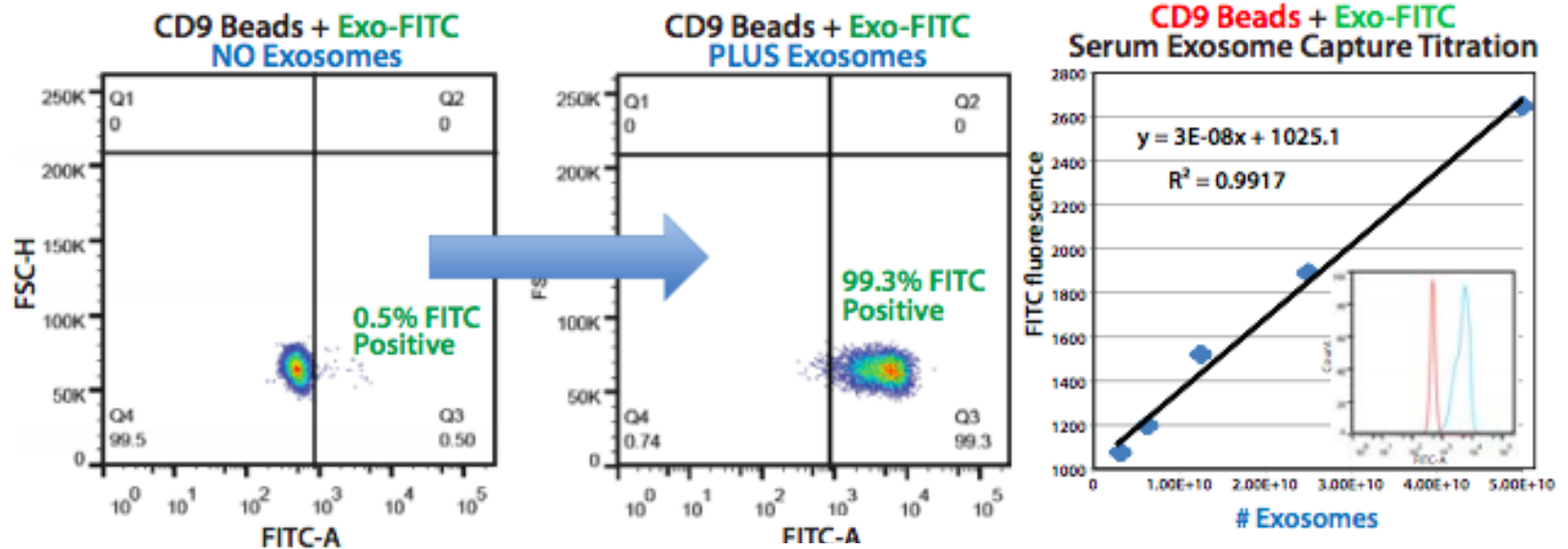
Marker-specific: subpopulation

- Affinity-based

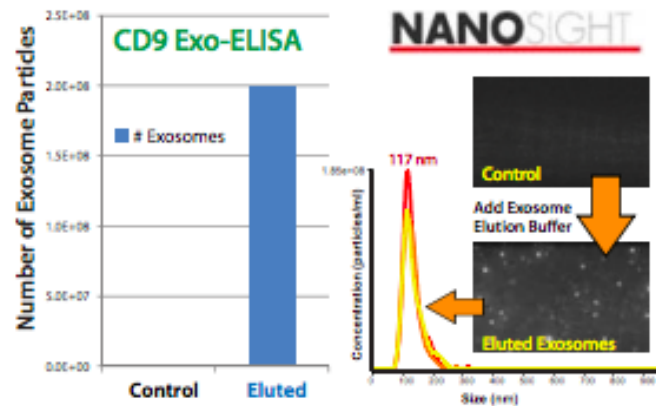
Exosome Surface Marker isolation : ExoFlow IP



Affinity Isolation: ExoFlow



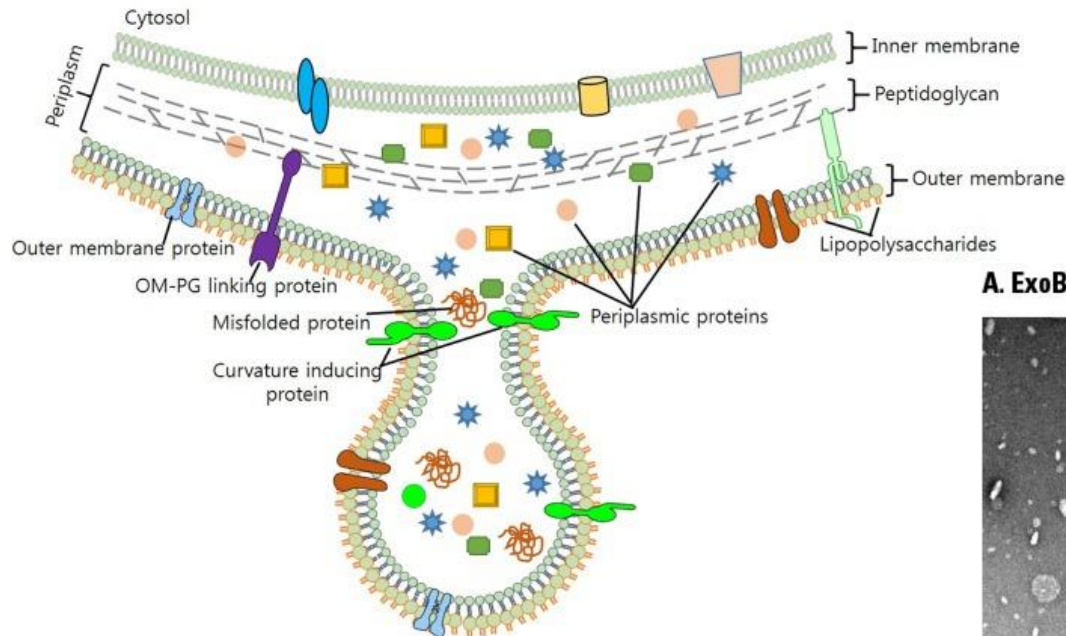
Obtain highly-purified and intact exosomes



Outer Membrane Vesicles

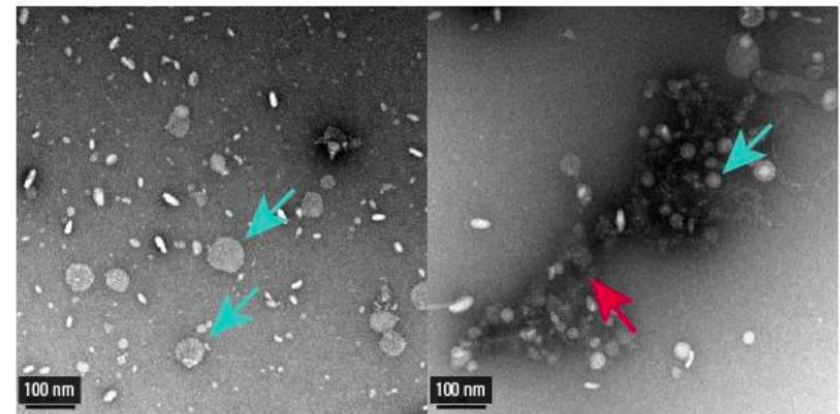
ExoBacteria™ OMV Isolation Kit

- Fast— go from cultured bacterial media to purified OMV in less than 1 hour
- Simple—easy-to-setup, innovative precipitation-free, ion-exchange chromatography system (containing a proprietary capture resin + gravity column)



A. ExoBacteria OMV Isolation Kit

B. Ultracentrifugation



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EV Characterization: MISEV

Minimal experimental requirements for definition of EVs

General characterization:

- ≥ 3 positive protein markers of EVs, being at least 1
 - a) transmembrane/lipid-bound protein
 - b) cytosolic protein
- ≥ 1 negative protein marker

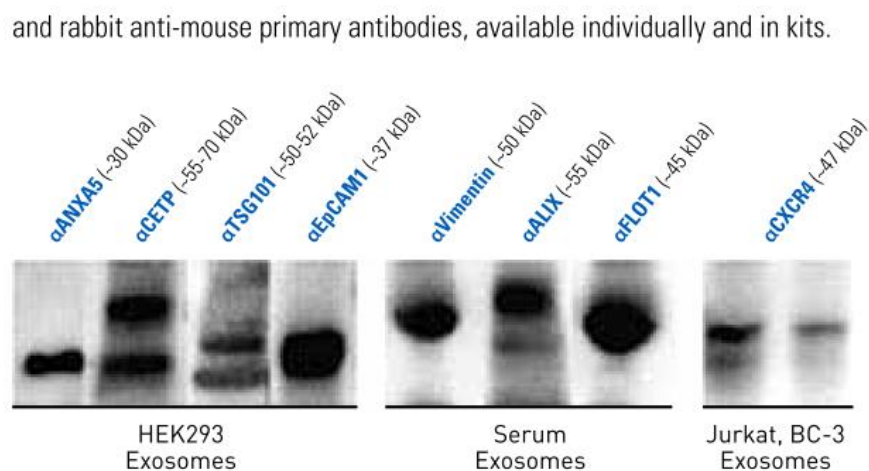
Characterization of single vesicles (Count & Size):

- Use 2 different but complementary techniques i.e:
 - a) electron or atomic force microscopy
 - b) single particle tracking (NTA)

Marker identification

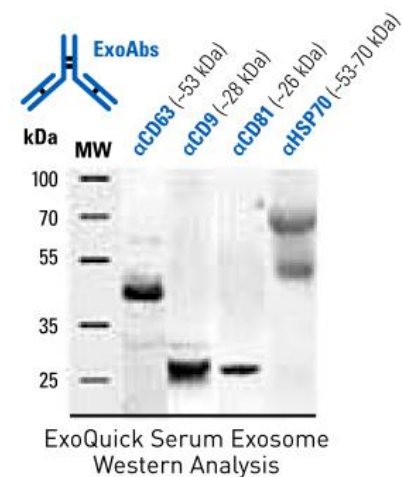
Antibodies

For affinity-based exosome detection, SBI offers validated rabbit anti-human and rabbit anti-mouse primary antibodies, available individually and in kits.



Additional validated exosome marker antibodies:

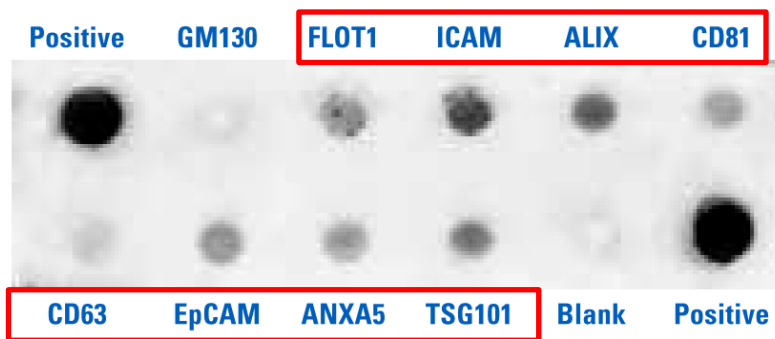
ANXA5 / CETP / TSG101 / EpCAM1 / Vimentin / ALIX / FLOT1 / CXCR4



General exosome marker antibodies:

CD63 / CD9 / CD81 / HSP70

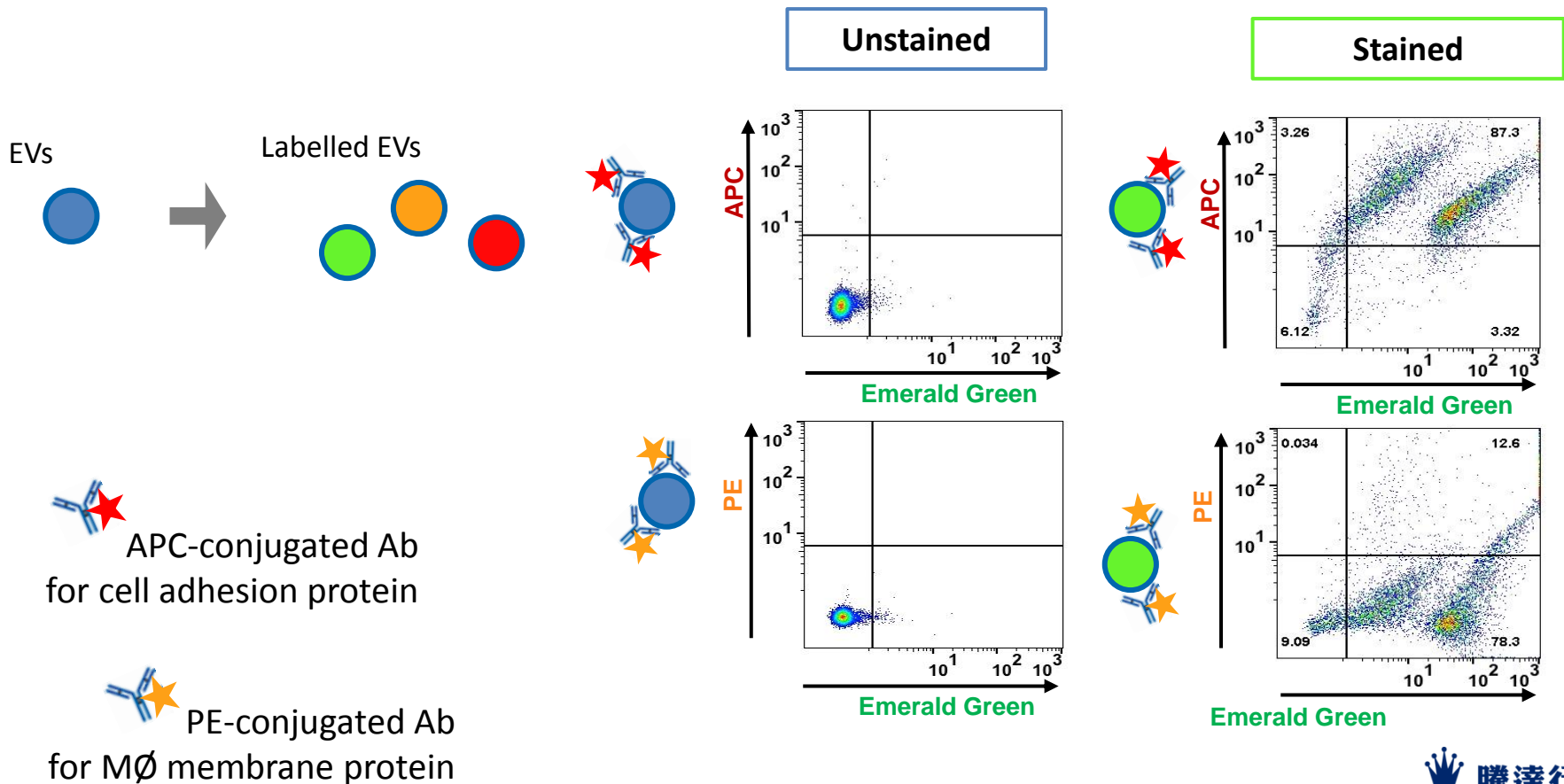
Exo-Check Antibody Array



⚡ Exo-Check array hybridized to 300 μ g of exosomal proteins from human HT1080 lung sarcoma cells. Exosomes were isolated from the growth media using ExoQuick-TC.

ExoFlow-ONE: Fluorescence-based EV Detection

- Specific fluorescence-based FACS detection of EVs, including exosomes
- Can be used on its own: near single EV resolution (based on the instrument)
- Can be combined with conjugated-primary antibody tagging

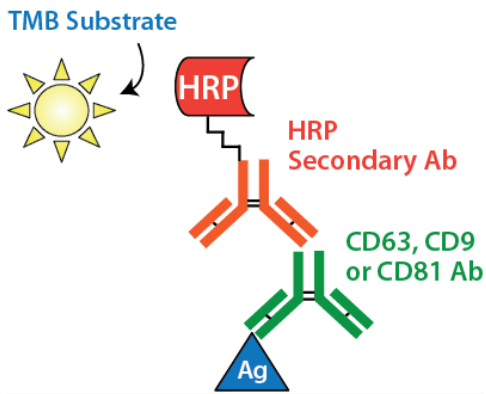


Quantitation & size distribution

Assay	Detection	EV size	EV count	SBI
Bradford/BCA	Protein determination	-	-	-
ELISA – EV marker	Antibody	-	+ subpopulation (if standard curve used)	Exo-ELISA Exo-ELISA ULTRA
AChE activity	Enzymatic reaction	-	+ (if standard curve used)	EXOCET FLUOROCET
Transmission Electron Microscopy (TEM)	Imaging	+	-	-
Nanoparticle Tracking Analysis (NTA)	Particle tracking	+	+	Exo-Glow NTA

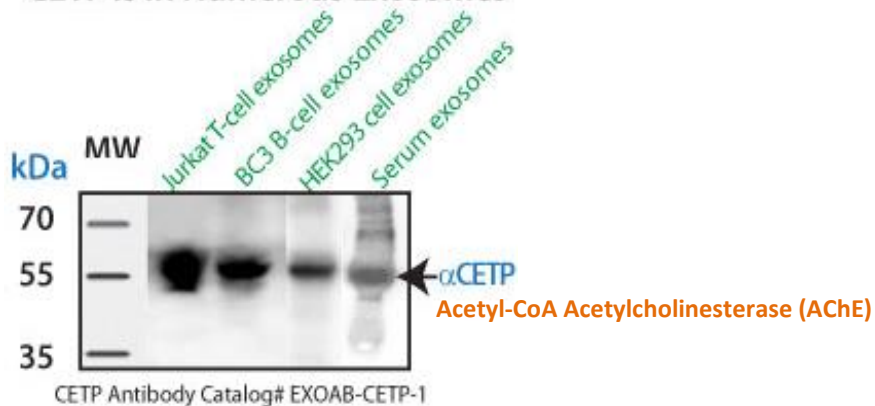
Exosome Quantification

Antibody-based : ExoELISA, ExoELISA-ULTRA



Activity-Based: EXOCET, FluoroCet

CETP is in Numerous Exosomes



Kit	ExoELISA-ULTRA CD63	ExoELISA	EXOCET	FluoroCet
Use	For fast and sensitive antibody-based detection of exosomes	For sensitive detection of exosomes when time is not limiting	For fast quantitation of extracellular vesicles with moderate sample input requirements	For the most sensitive detection of extracellular vesicles with very low sample input requirements
Chemistry	Enzymatic, antibody	Enzymatic, antibody	Enzymatic, colorimetric	Enzymatic, Fluorescent
Protocol Time	4 hours (no overnight incubation)	24 hours	20 min	20 min
Input Amount	1-200 µg protein equivalent	>500 µg protein equivalent	50 µg protein equivalent	<1 µg protein equivalent

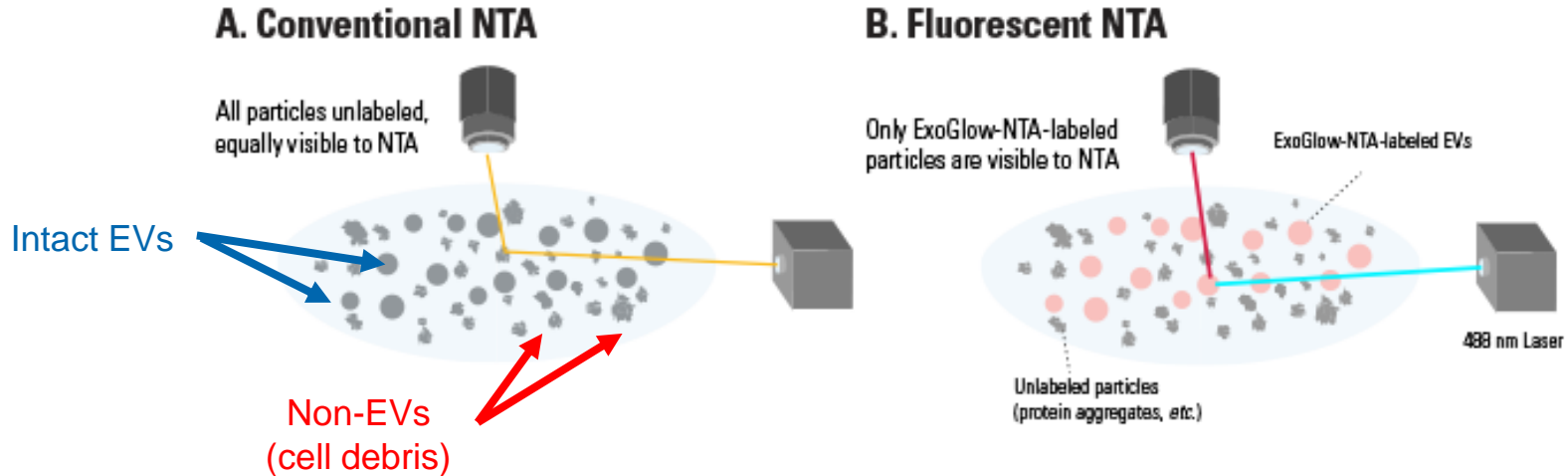
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AChE activity	Enzymatic reaction	-	+ (if standard curve used)	EXOCET FLUOROCET
Transmission Electron Microscopy (TEM)	Imaging	+	-	-
Nanoparticle Tracking Analysis (NTA)	Particle tracking	+	+	Exo-Glow NTA

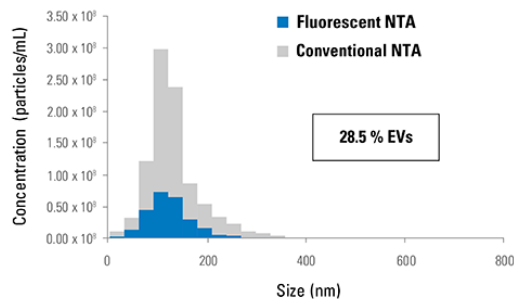
EV Size Distribution & Quantitation: NTA

- Conventional NTA allows
 - Count of EVs/mL
 - EV size distribution

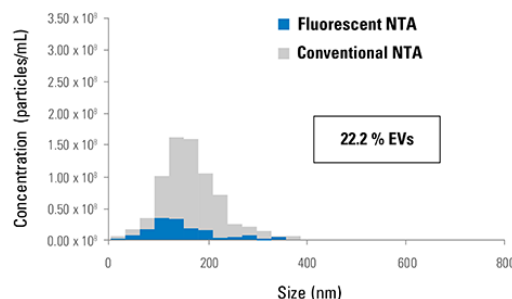
- Fluorescent NTA (ExoGlow™-NTA Fluorescent Labeling Kit) allows
 - Discrimination of intact Evs
 - More accurate & reliable : Count of EVs/mL, EV size distribution



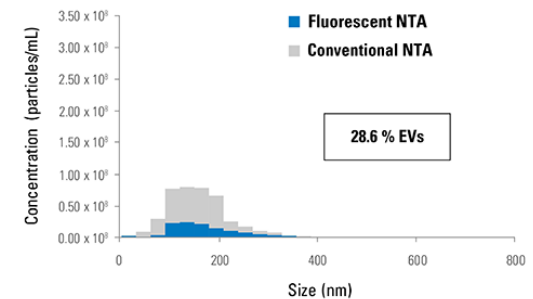
A. ExoQuick



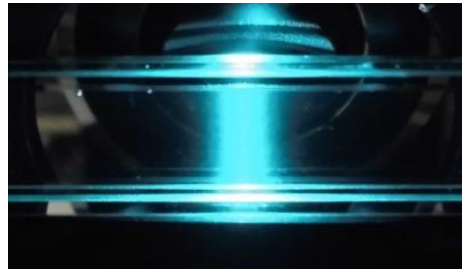
B. Ultracentrifugation + wash



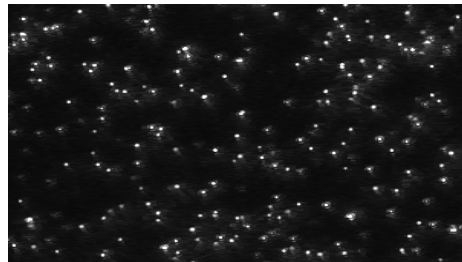
C. Column-based



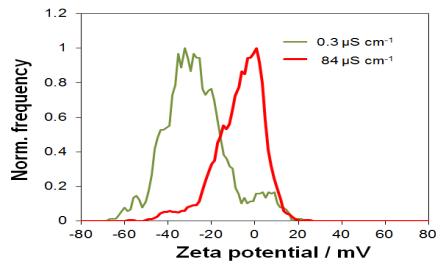
Nanoparticle Tracking Analysis-ZetaView



Inject



Visualize



Measure



Size

Count

**Zeta
Potential**

Fluorescence

Exosome Research Tools

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- [Exo-FBS](#)
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[View Products](#)

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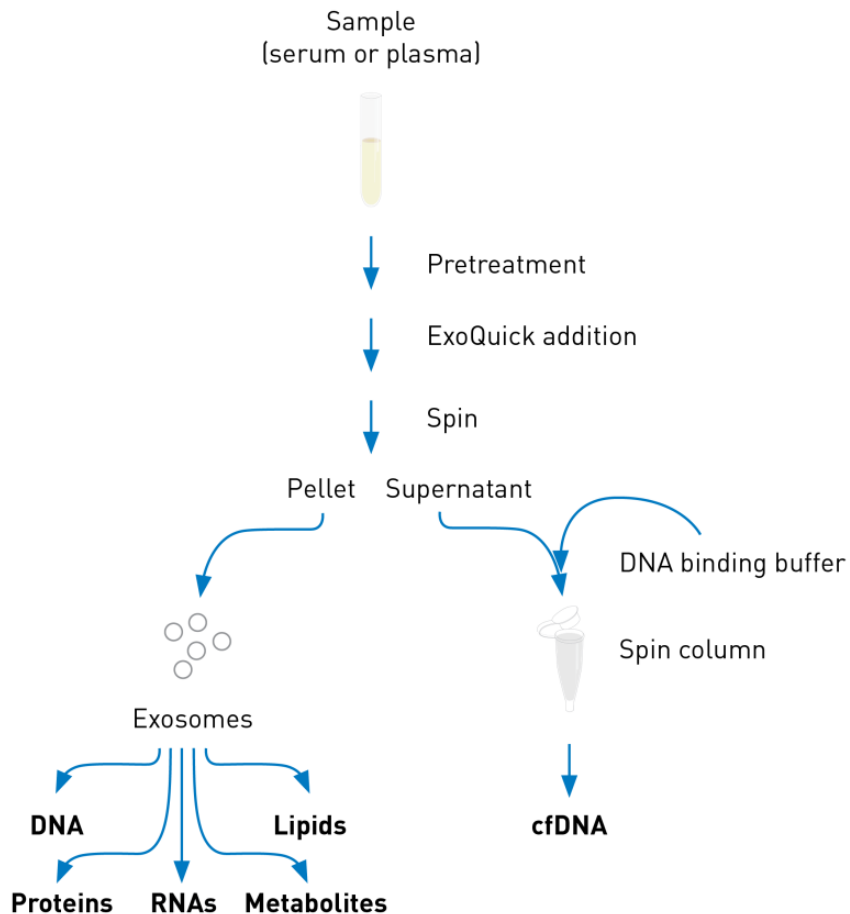
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cfDNA and exosomal DNA Isolation

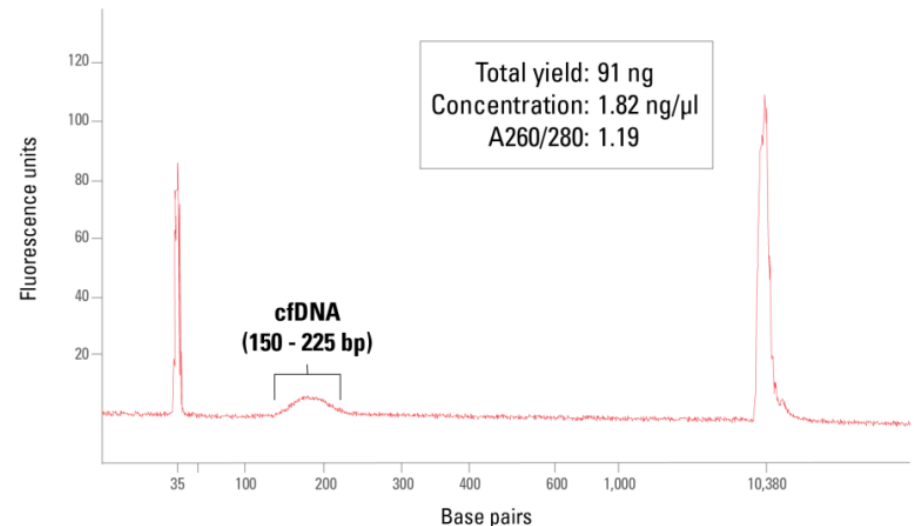


XCF™ COMPLETE Exosome & cfDNA Isolation Kits

- No sample splitting
- Enables simultaneous detection of **cfDNA and exosomal biomarkers**
- Compatible with downstream applications, e.g. qPCR, NGS

Good Quality and Yield of cfDNA

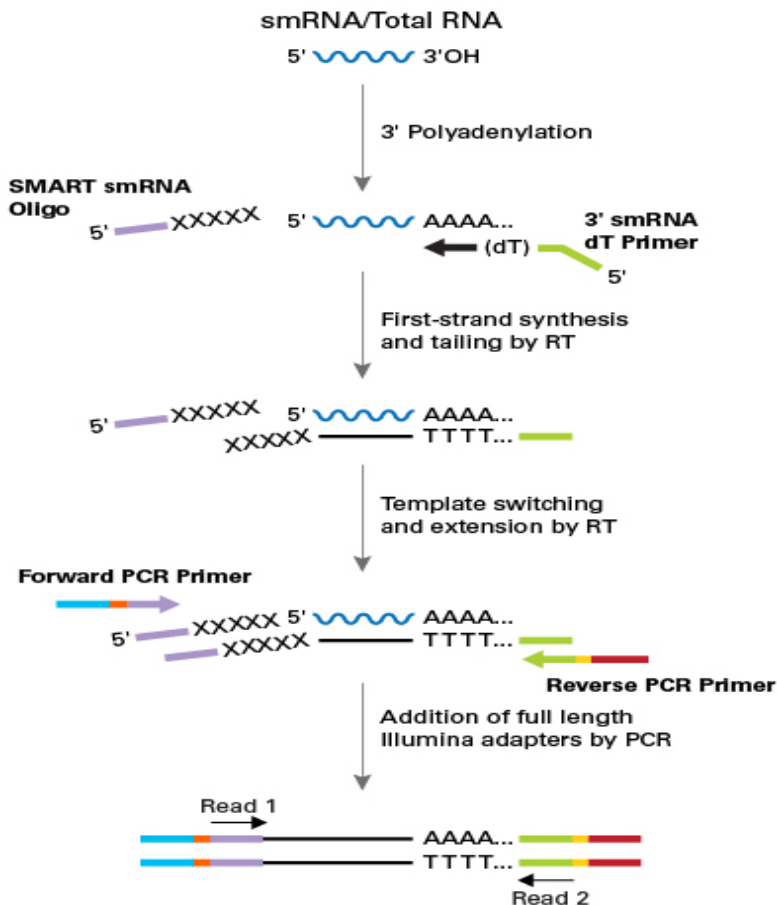
B.



Exosome miRNA Analysis

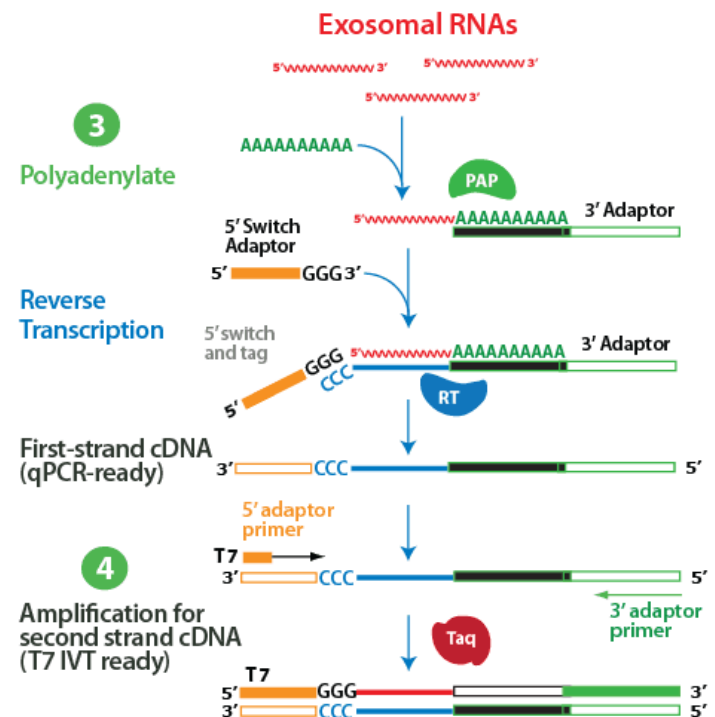
Exosomal RNA-Seq- SMARTer® smRNA-Seq Kit for Illumina®

- Flexible sample inputs - 1 ng–2 µg of total RNA or enriched small RNA
- Illumina-ready sequencing libraries



Exosomal RNA qPCR-SeraMir Exosome RNA Purification Kit

- Increase sensitivity - amplify exoRNAs for qPCR
- Gain more data - use T7 IVT amplified sense exoRNAs for microarrays and NextGen sequencing

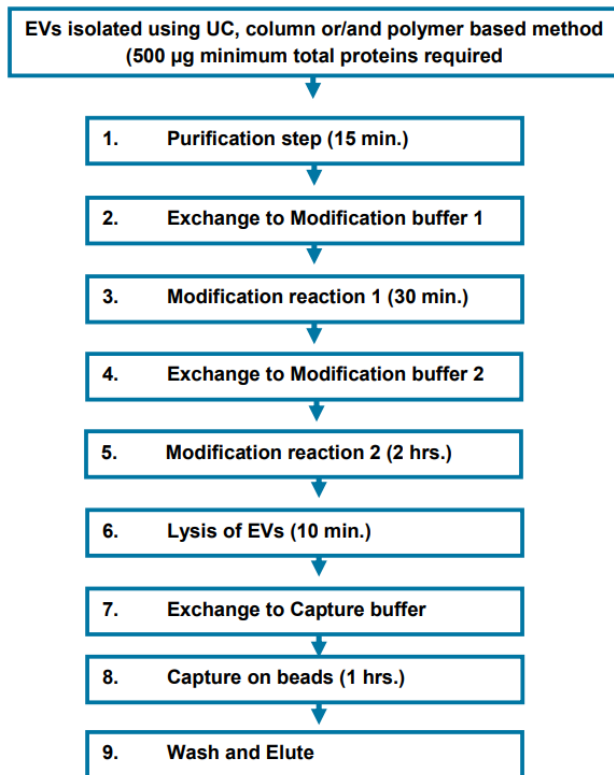


Exosome protein Analysis

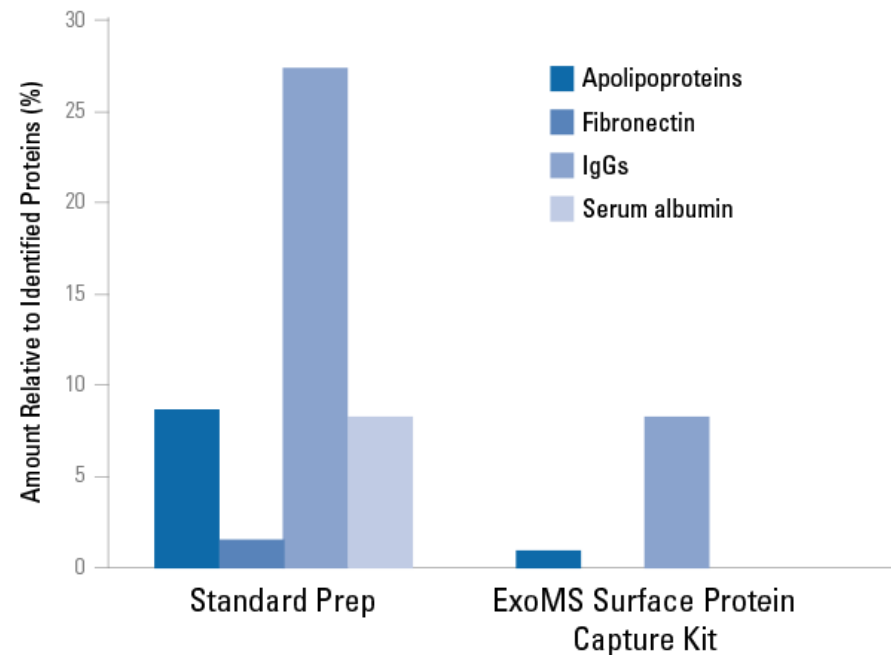
Exosomal Proteomics-Mass Spec

ExoMS™ Surface/Total Protein Capture Kit

- The only commercially available kit that specifically captures exosome surface membrane and total proteins
- Go from isolated sample to LC/MS in less than 5 hours
- two-step biotinylation and bead capture method



Low residual protein carryover



Exosome standards

- Lyophilized and purified exosomes which are purified by TFF, SEC and microfiltration
- Standardized positive controls for different analysis, e.g protein marker, nucleic acid

Cat Code	Source
Lyophilized Exosome Standards from Human Biofluids	
HBM-PEP	Lyophilized Exosome from Human Plasma of healthy donors
HBM-PES	Lyophilized Exosome from Human Serum of healthy donors
HBM-PEU	Lyophilized Exosome from Human Urine of healthy donors
HBM-PESL	Lyophilized Exosome from Human Saliva of healthy donors
Lyophilized Exosome Standards from Cell Culture Media	
HBM-COLO1	Lyophilized Exosomes from COLO1 cell line (Human colon carcinoma)
HBM-MM1	Lyophilized Exosomes from MM1 cell line (Human melanoma)
HBM-BLCL	Lyophilized Exosomes from BLCL21 cell line (EBV transformed lymphoblastoid B cells)
HBM-HCT	Lyophilized Exosomes from HCT116 cell line (Human colon carcinoma)
HBM-U87	Lyophilized Exosomes from U87 MG cell line (Human glioblastoma astrocytoma)
HBM-SK	Lyophilized Exosomes from SK-N-SH cell line (Human neuroblastoma)
HBM-PC3	Lyophilized Exosomes from PC3 cell line (Human Prostate adenocarcinoma grade IV)
HBM-BPH	Lyophilized Exosomes from BPH-1 cell line (Human being prostatic hyperplasia)
HBM-DAUDI	Lyophilized Exosomes from DAUDI cell line (Human Burkitt Lymphoma)
HBM-A549	Lyophilized Exosomes from A549 cell line (Lung carcinoma)
HBM-K562	Lyophilized Exosomes from K-562 cell line (Pleural effusion, leukemia chronic myelogenous)
HBM-B16F10	Lyophilized Exosomes from B16F10 mouse cell line (mouse melanoma cell line)
Lyophilized Exosome Standards are sold in vials containing 100 µg or 30 µg of total protein (Particles/ml > 1x10 ¹⁰) HBM can provide, upon request, Exosome Standards and Cell Lysates from more than 100 different tumor cell lines. Contact us at info@hansabiomed.eu	

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- [ExoMS Surface Protein Capture Kit \(Serum/Plasma EVs\)](#)

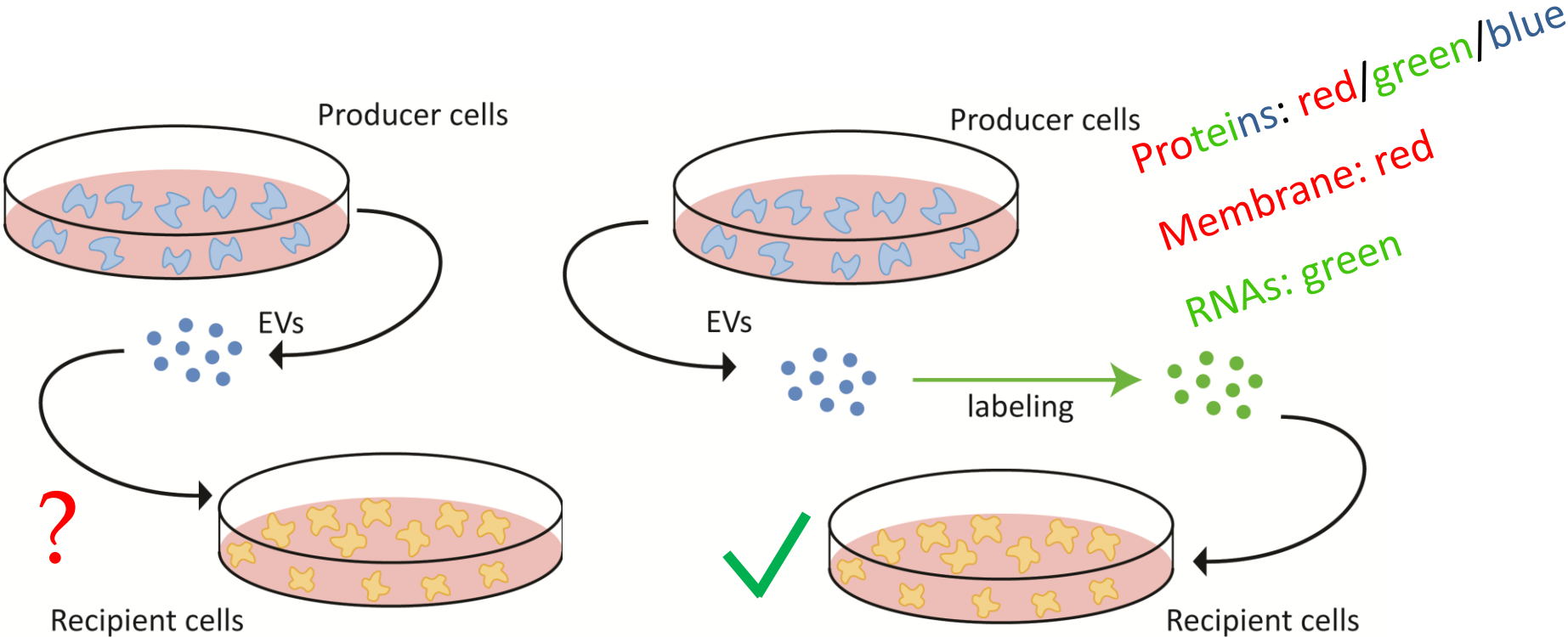
[View Products](#)

Exosome Engineering

- [XPack MSCV-XP-GFP-EF1 \$\alpha\$ -Puro Expression Lentivector](#)
- [XPack CMV-XP-MCS-EF1 \$\alpha\$ -Puro Cloning Lentivector](#)
- [Exo-Fect Exosome Transfection Kit](#)

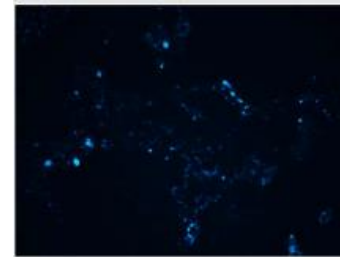
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Confirming Exosome Uptake in Recipient Cells

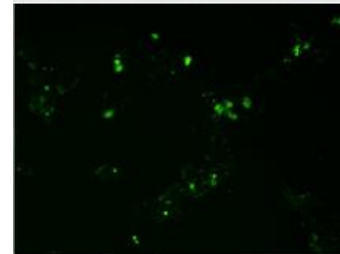


ExoGlow -Protein/RNA/Membrane EV Labeling Kit

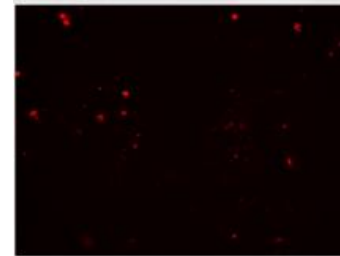
- Ability to track and localize EVs
- **ExoGlow Protein**: Membrane permeable, non-fluorescent dye is activated upon EV internalization by enzymatic conversion to a fluorescent moiety
- **ExoGlow RNA**: mRNA detection probe, polyT-fluorescent moiety
- **ExoGlow Membrane** : highly specific membrane sensor, requires a charge potential in order for them to fluoresce



ExoGlow P- **Blue**



ExoGlow P- **Green**



ExoGlow P- **Red**

Exosome Research Tools

Exosome Isolation

- [ExoQuick-ULTRA EV Isolation Kit](#)
- [Exo-FBS](#)
- [ExoQuick-TC EV Isolation Kit](#)

[View Products](#)

Exosome Detection

- [ExoCheck Exosome Antibody Array](#)
- [ExoFlow-ONE EV Labeling Kit \(Emerald\)](#)
- [ExoFlow-ONE EV Labeling Kit \(Garnet\)](#)

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Exosome Quantitation

- [ExoELISA-Ultra Complete Kit \(for CD63 Detection\)](#)
- [ExoELISA-Ultra Complete Kit \(for CD81 Detection\)](#)
- [FluoroCet Exosome Quantitation Kit](#)

[View Products](#)

Exosome Labeling

- [ExoGlow-Protein EV Labeling Kit \(Green\)](#)
- [ExoGlow-Membrane EV Labeling Kit](#)
- [ExoGlow-RNA EV Labeling Kit](#)

[View Products](#)

Biomarker Profiling

- [Human Complete SeraMir Exosome RNA Amplification and Profiling Kit](#)
- [Purified Exosomes from Human Serum \(Healthy Donors\)](#)
- [ExoMS Surface Protein Capture Kit \(Serum/Plasma EVs\)](#)

[View Products](#)

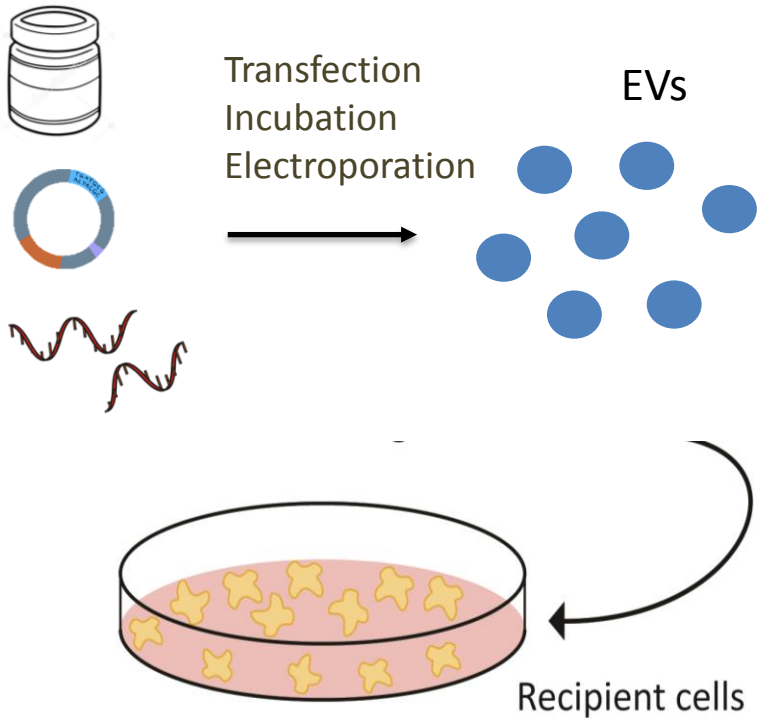
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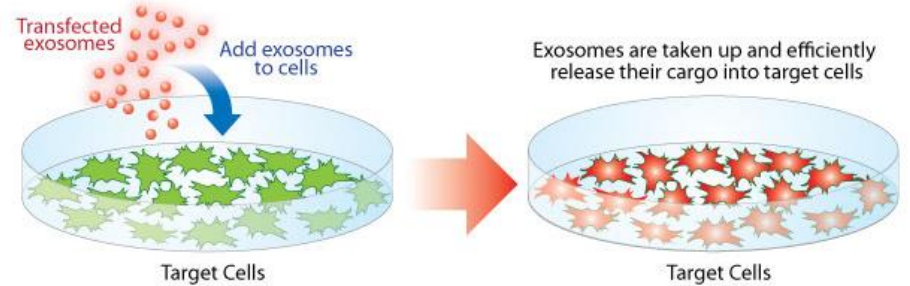
[View Products](#)

Therapeutic Drug Carriers

- Load chemotherapeutic agents, siRNAs, and miRNAs

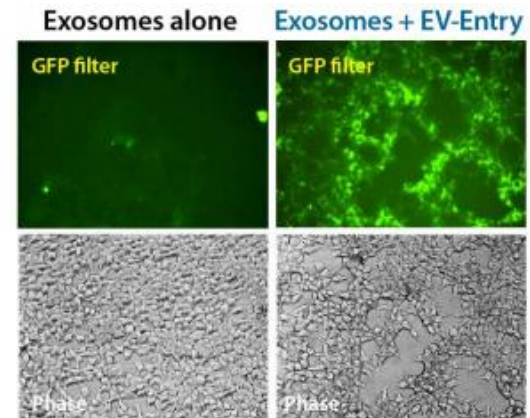


- Novel nucleic acid transfer agent
Transfect exosomes and watch them deliver cargo to cells

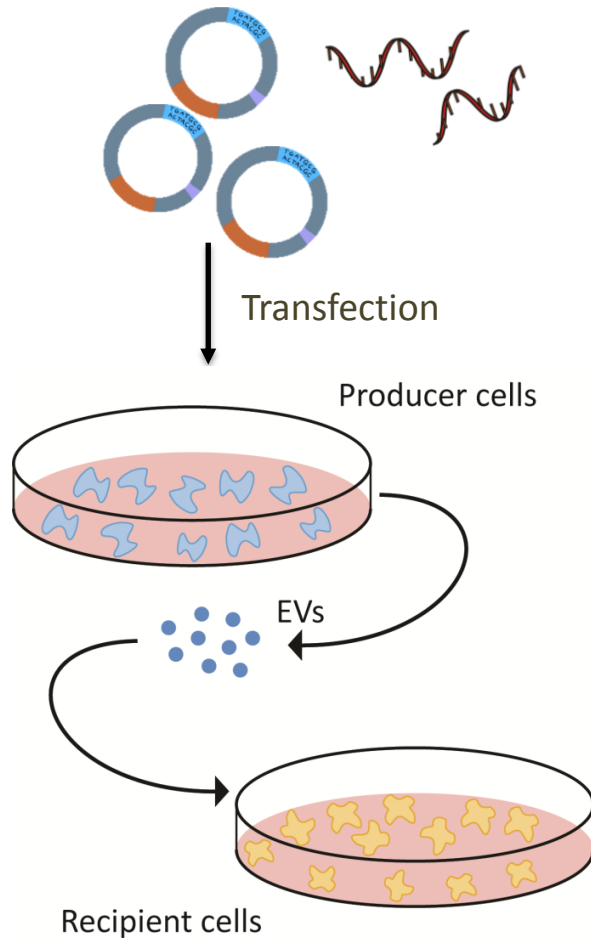


EV-Entry System for Exosome Delivery

- Boosts the delivery of both RNA and protein cargo



Therapeutic Drug Carriers



- Package active proteins, nucleic acid, and other active molecules into exosomes, where cells are transfected with an engineered effector-expressing vector.

1. Targeting System

- XSTAMP

2. Packaging System

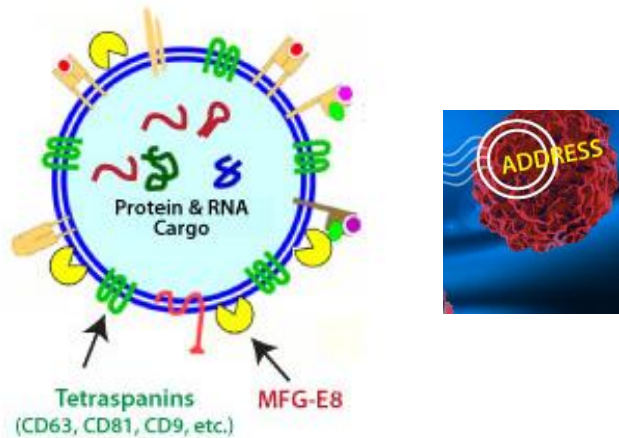
- XMIR/AXMIR and XPACK

Targeting Systems

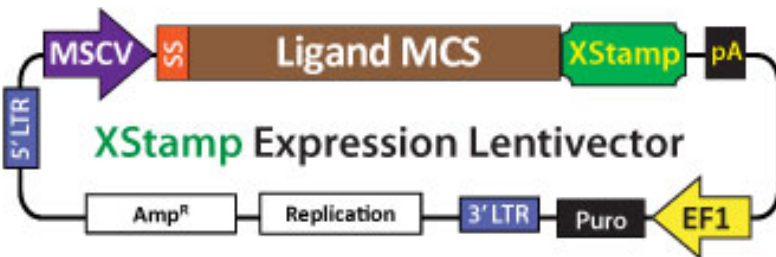
XStamp™ Exosome Targeting

XStamp

- Engineer exosome surfaces for protein ligand



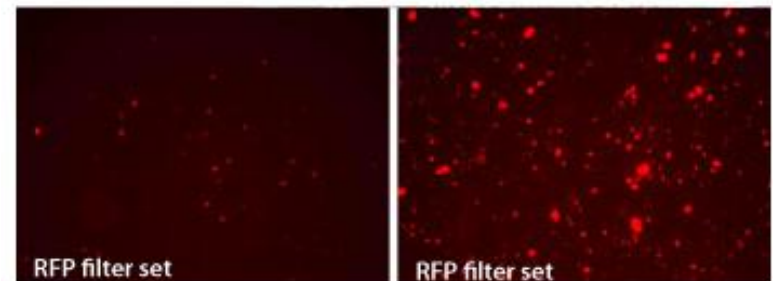
MFG-E8 Localizes to Exosome Surfaces (C1C2 domain)



Add labeled XStamp-Motilin Exosomes to Breast cancer or Colon Cancer Cells

MDA-MB-231 Breast Cancer Cells

HT-29 Colon Cancer Cells



(motilin receptor negative)

(motilin receptor positive)

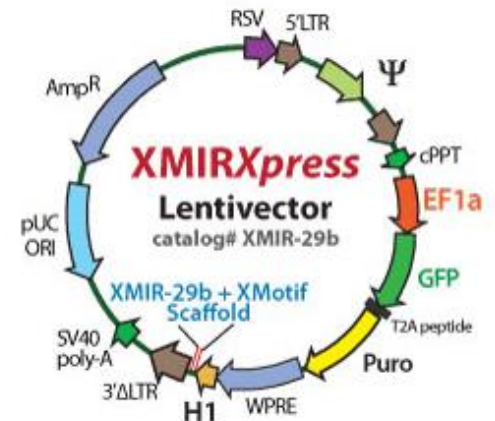
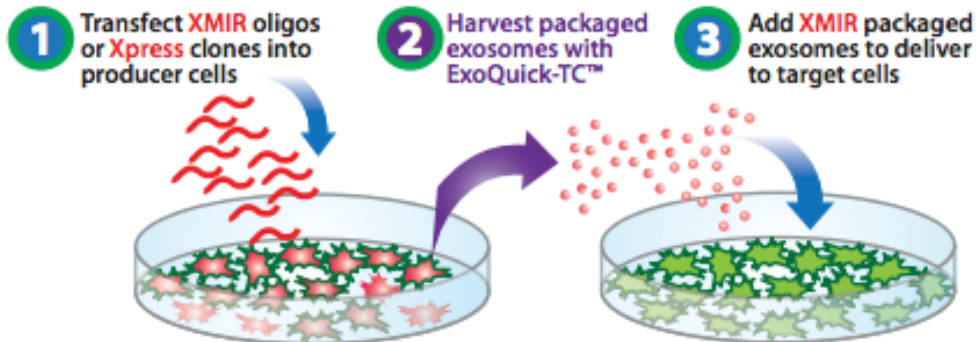
Packaging Systems

XMIR, AXMIR and XMIRXpress exosome RNA packaging systems

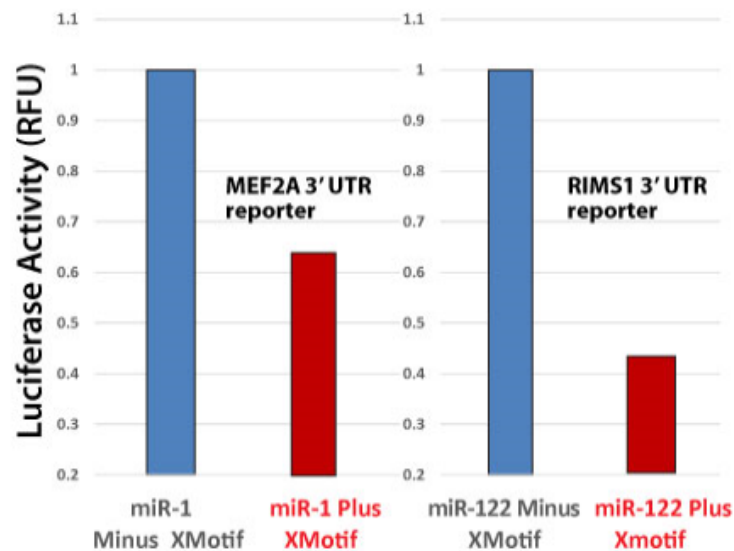
XMIR/AXMIR



- Package miRNAs into Exosomes
- “XMotif RNA Sequence”



Packaged XMIR-1 and XMIR-122 delivery to 3' UTR reporter cells

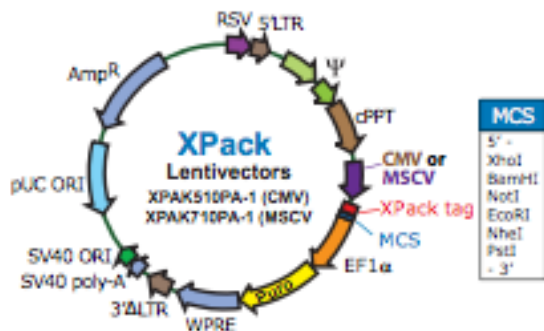


Packaging Systems

XPack™ Exosome Protein Engineering

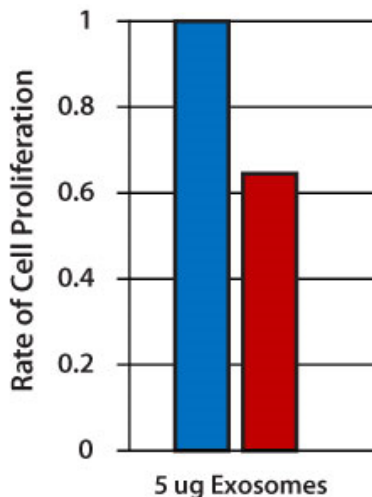
- Tag fusion proteins for incorporation into secreted exosomes
- “XPack peptide sequence”

XPACK



XPack Lentivector Features

- CMV or MSCV promoter options
- Built-in XPack N-terminal tag
- EF1-Puromycin selection cassette
- High titer lentivirus backbone



CDKN18: a cell cycle inhibitor protein

■ HEK-293 Exosomes ■ XPack-CDKN1B Exosomes



Thank you for your attention