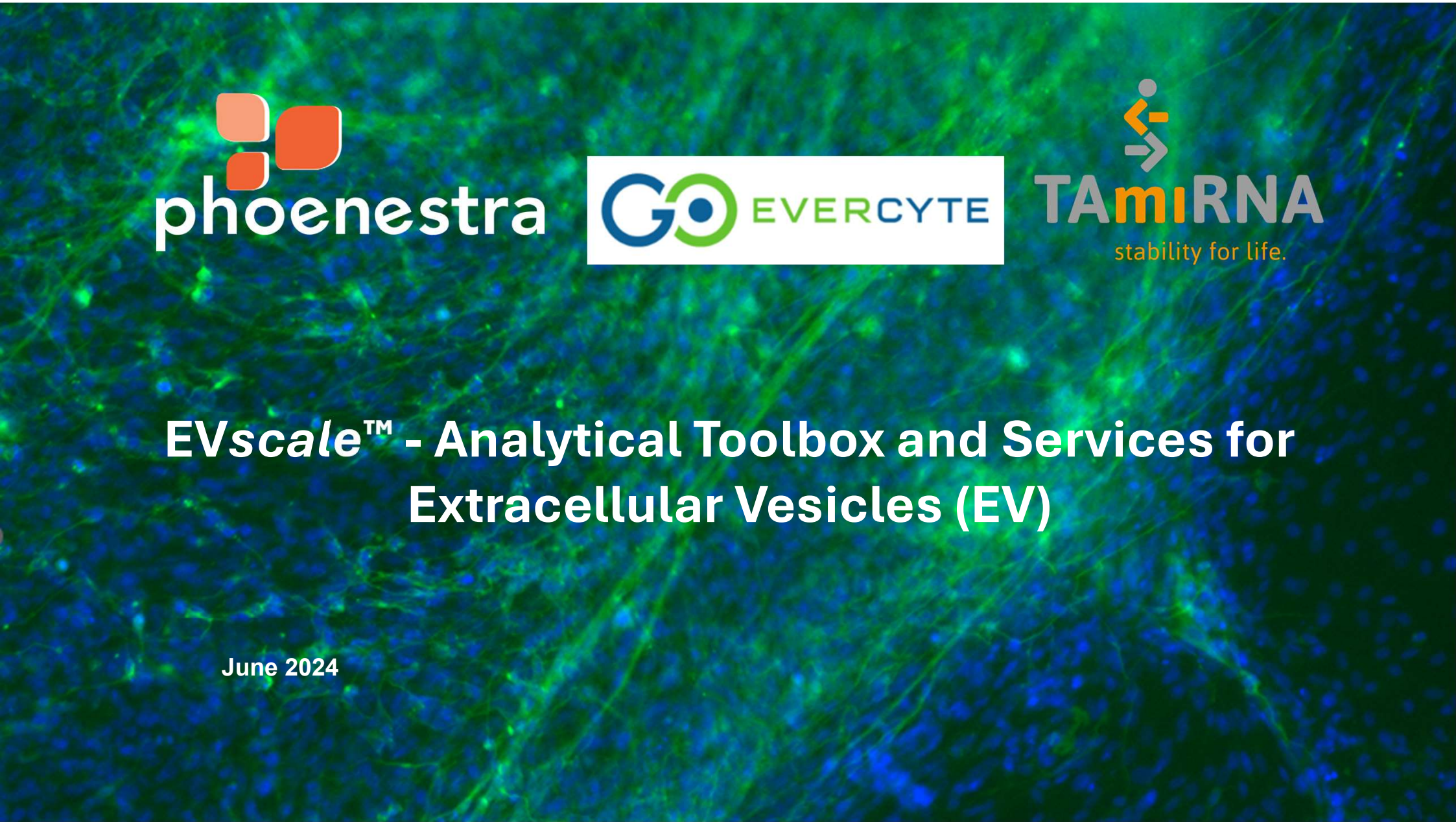
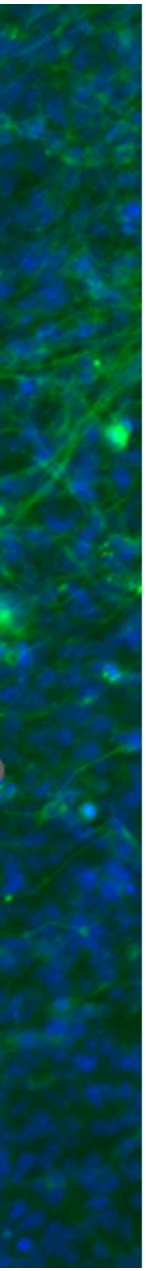
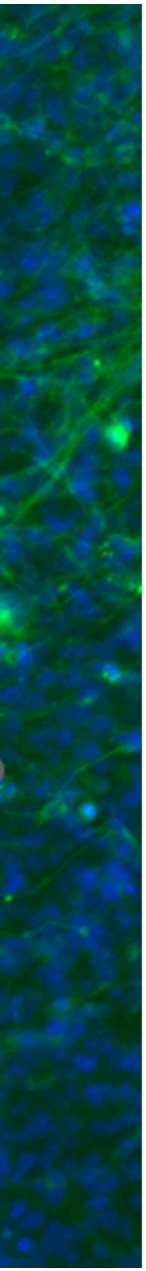

phoenestra
EVERCYTE
TAmiRNA
stability for life.
EVscale™ - Analytical Toolbox and Services for Extracellular Vesicles (EV)
June 2024



EV Characterization Toolbox and Services (1/3)

Particle Analysis	Assay Result
Nanoparticle Tracking Analysis (NTA)	Particle number, median particle size
NanoFCM, unstained	Particle Number, median particle size
NanoFCM, staining	Lipid vs. non-lipid particles (%) Protein markers (% marker positive particles) Lipid + Protein Marker: dual color staining of both lipid and protein marker
Pierce™ BCA Assay	Total protein concentration
Cryo-Electron Microscopy (outsourced)	Identity and integrity of EV particles



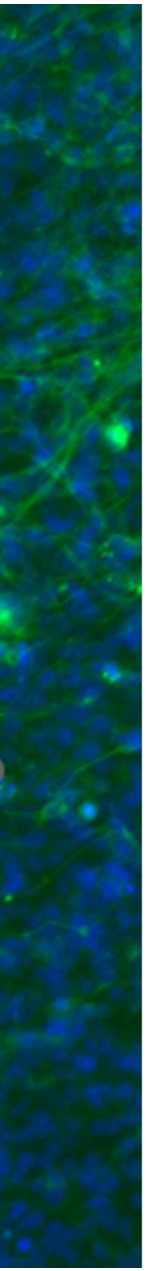
EV Characterization Toolbox and Services (2/3)

Functional Biological Assays*	Assay Result
Anti-inflammatory assay	% reduction of inflammatory signal (Nitric Oxide secretion)
Anti-fibrotic assay	% reduction of expression of fibrotic protein (α-smooth muscle actin)
For more bioassay options please visit: www.evercyte.com	

* In collaboration with Evercyte

RNA Profiling**	Assay Result
RNAseq	Total RNA content
miRNA analysis	Full miRNA profile and sequencing
For more options on RNA profiling, - omics and Biomarker development please visit: www.tamirna.com	

** In collaboration with TAmiRNA



EV Characterization Toolbox and Services (3/3)

Quality Control	Assay Result
Endotoxin / LAL (Ph.Eur. 2.6.14 / USP <85>) (outsourced)	Endotoxin level (E.U./mL or mg)
Sterility (Ph.Eur. 2.6.1 / USP <71>) (outsourced)	Colony forming units per mL
MycoStrip Mycoplasma Test	Detection of 16S rRNA of most common mycoplasma species

Nanoparticle Tracking Analysis (NTA)

Sample Parameters
Sample Name: 230214_DSP24-0053.8
Comment: PS100nm 1:250,000, Sample Remarks0:
Sample Remarks1:
Sample Remarks2:
Electrolyte:
Temperature: 22.77 °C sensed
pH 7.0 entered
Conductivity: 0.00 µS/cm entered

Measurement Parameters
Cell S/N: NTA

Measurement Mode: Size Distribution 2 Cycles
11 Positions

Result (sizes in nm)

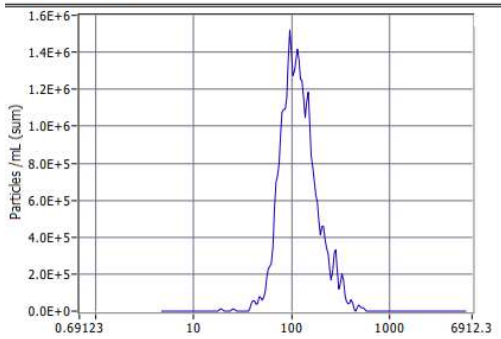
	Number	Concentration	Volume
Median (X50)	113.3	113.3	239.3
Span	64.6	64.5	118.2

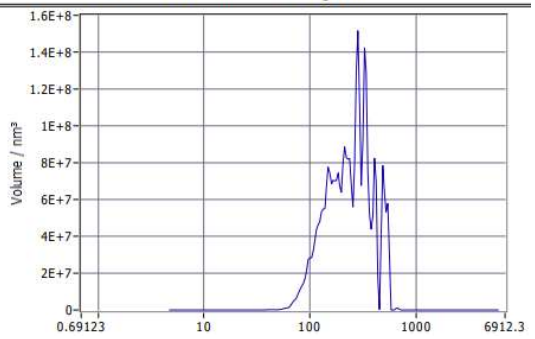
Concentration: 3.8E+7 Particles / mL
Dilution Factor: 1000
Original Concentration: 3.8E+10 Particles / mL

Particle concentration

Quality
Average Counted Particles per Frame: 106
Number of Traced Particles: 1466

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 30





Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
102.8	1.3E+6	92.9	100.0

X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	72.0	72.0	118.0
X50	113.3	113.3	239.3
X90	208.7	208.7	415.9
Span	1.2	1.2	1.2
Mean	131.9	131.9	259.8
StdDev	64.6	64.5	118.2

Particle size

Nanoscale Flow Cytometry – NanoFCM unstained

nanofcm

Comprehensive Bio-Nanoparticle Analysis
Nano-Flow Cytometry

Size & Concentration Report

DSP24-0161 1in100

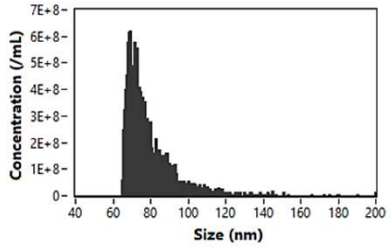
Data File 20240417 DSP24-0161 1in100 7.nfa Population Total

SN: FNAN30E23091222
Software: V2.0
Sample Pressure: 1,0Kpa

Laser: 8/50 mW 488 & 20/100 mW 638
SS Decay: 10%
Threshold/sub: 152,6 13,3 41,5 1/0 0 0 0
Min Width: 0,3 ms

Total Size Information

All Events	2840	
Gating Events	2840	
% of all	100,00	
Median	74,8	nm
Mean	79,1	nm
Std Dev.	13,9	nm



Particle size

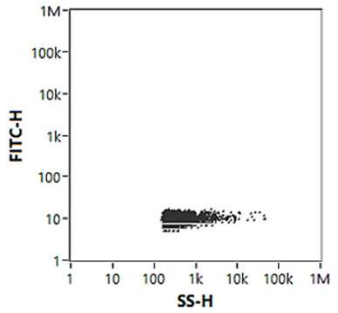
Particle size distribution histogram

Total Concentration Information

	Particle Number	Dilution Factor
STD	3668	100
Blank	3	——
Sample	2843	100

STD Con.	2,17E+10	Particles/mL
Sample Flow Rate	16,9	nL/min
Sample Con.	1,68E+10	Particles/mL

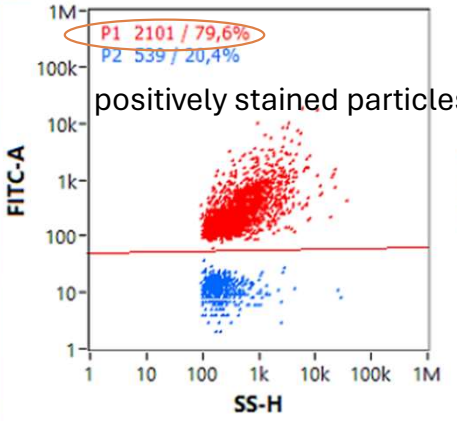
Corrected Ratio: 2840/2840 100,0%



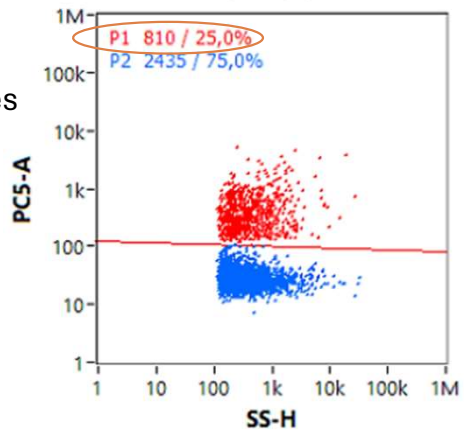
Particle concentration

Nanoscale Flow Cytometry – NanoFCM EV staining

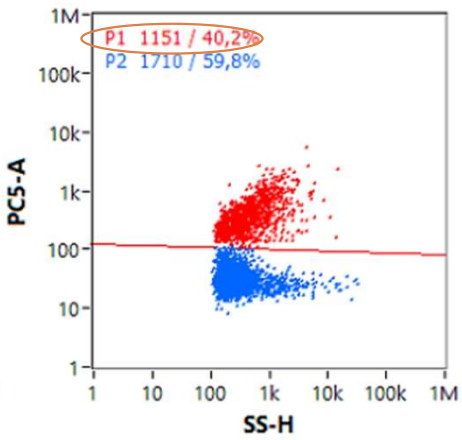
MemGlow



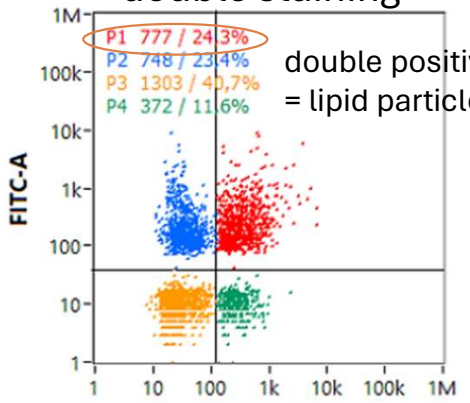
CD63



CD73



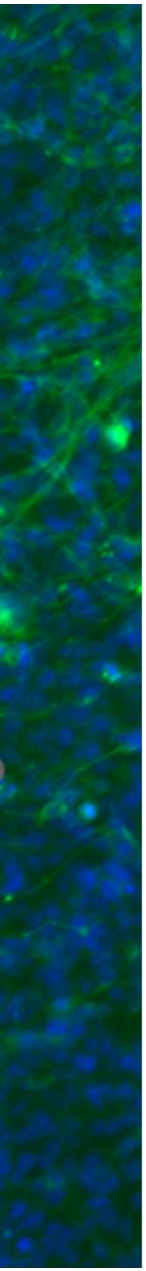
double staining



double positive particles
= lipid particles carrying the protein marker

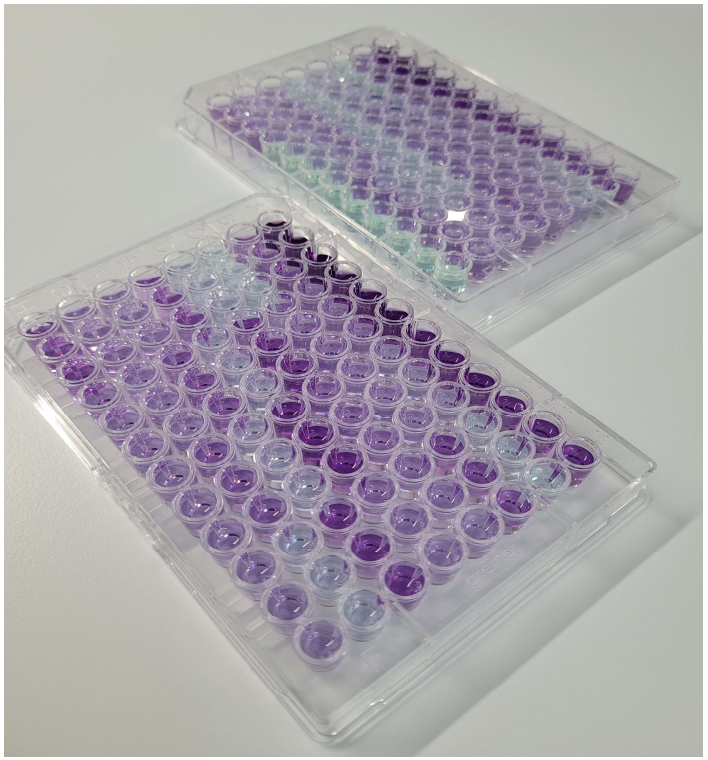
Currently established markers:

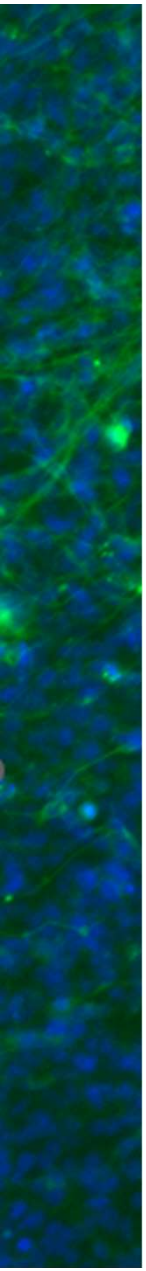
Marker Class	Marker
Tetraspanins	CD9
	CD63
	CD81
MSC surface proteins	CD44
	CD73
	CD90
	CD105
	CD146
Cytokines	IL1-RA
Mitochondria	Mitotracker
Lipids	MemGlow



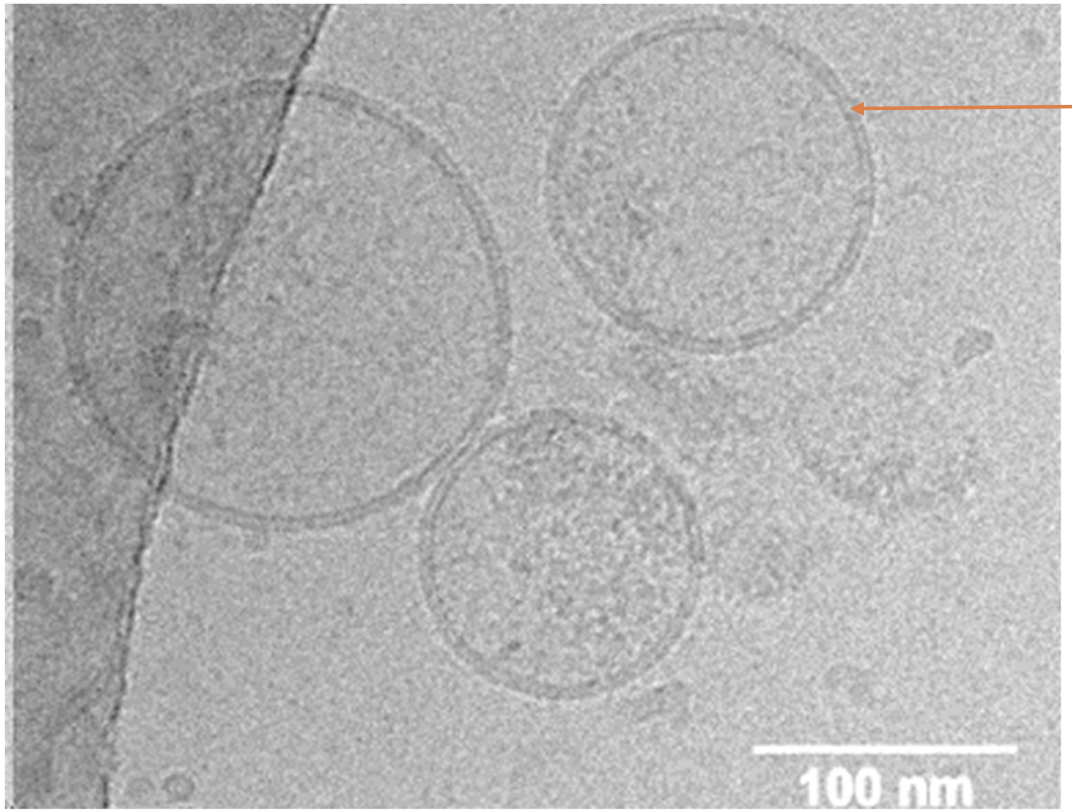
BCA (bicinchoninic acid) assay

- Total protein concentration determination
- Range: 25 – 2000 µg/ml
  (lower limit with lysis: 250 µg/ml)
- 2-step reaction → formation of intense purple-coloured complex with a linear absorbance at 562 nm with increasing protein concentrations

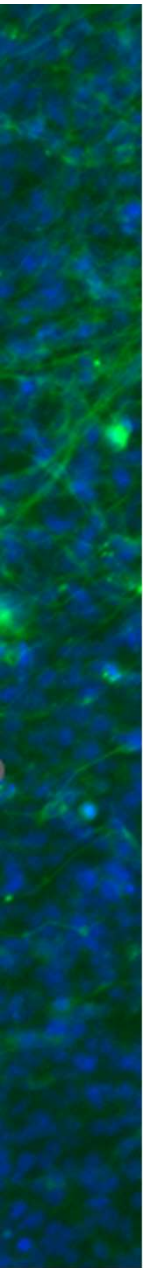




Cryo Electron Microscopy (CEM)



Double lipid layer



MycoStrip™ Mycoplasma Detection

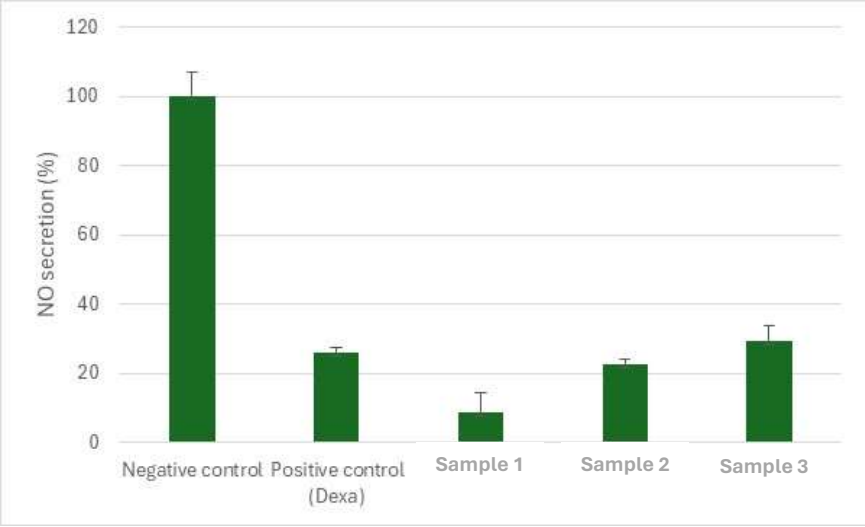
- Isothermal PCR of sample
  - amplification of 16S rRNA for the most common Mycoplasma species in cell culture
- Visualization on immunochromatographic strip (right)

Functional Biological Assays

Testing for anti-inflammatory (AI) activity of EV preparations

- Mouse Macrophage-based
- LPS triggered NO-secretion
- Dexamethasone as positive control

Developed and performed by EVERCYTE forever is just enough.

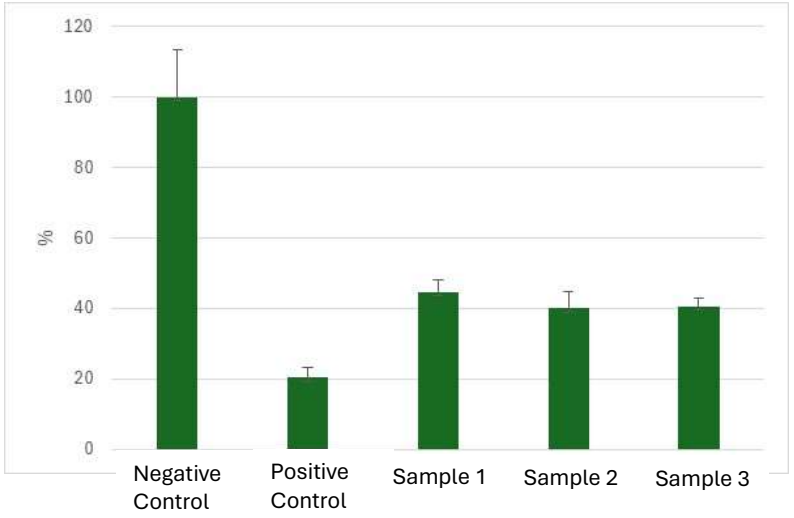


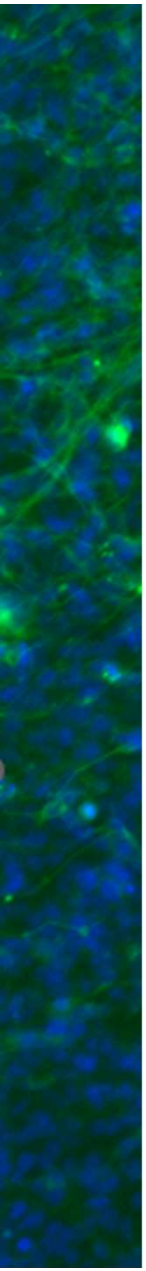
Functional Biological Assays

Testing for anti-fibrotic (AF) activity of EV preparations

- Fibroblast-based
- TGF-β-triggered α-SMA expression
- Kinase inhibitor PP2 as positive control

Developed and performed by





RNA Profiling

- Total RNA contents
- miRNA contents
- Heat maps
- Statistical analysis
- Analysis of selected miRNAs and miRNA patterns

Developed and performed by

TAmiRNA
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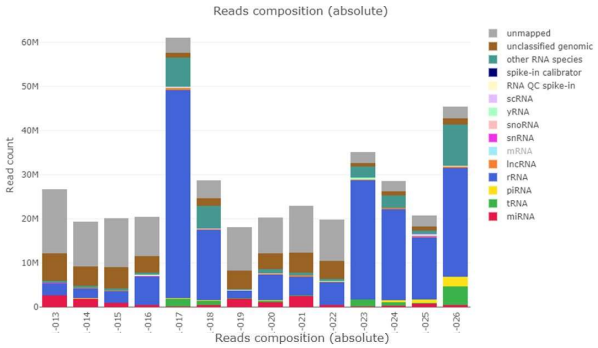

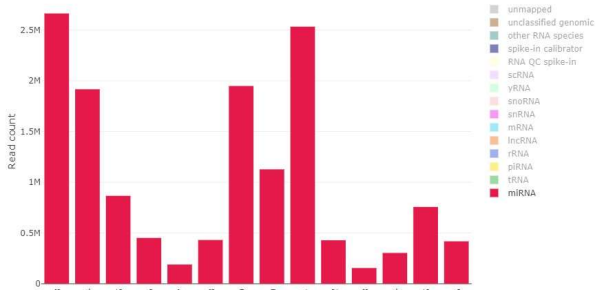

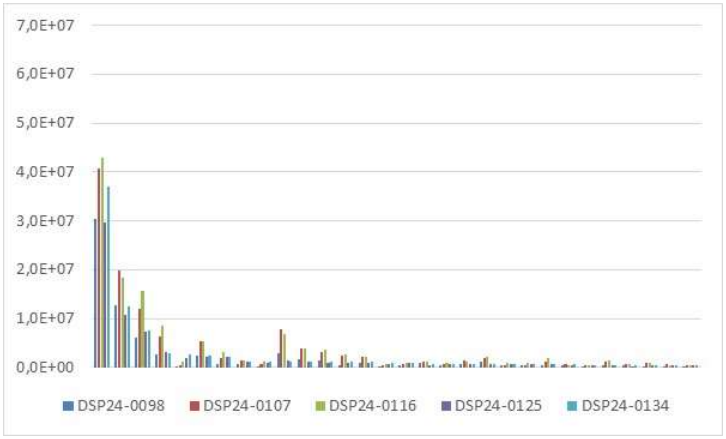

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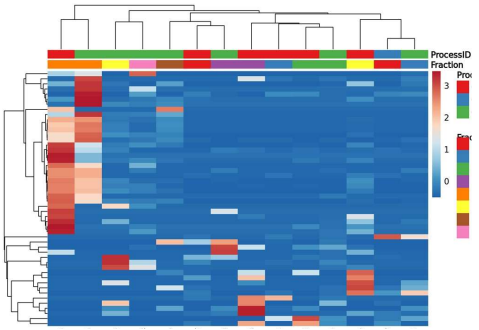
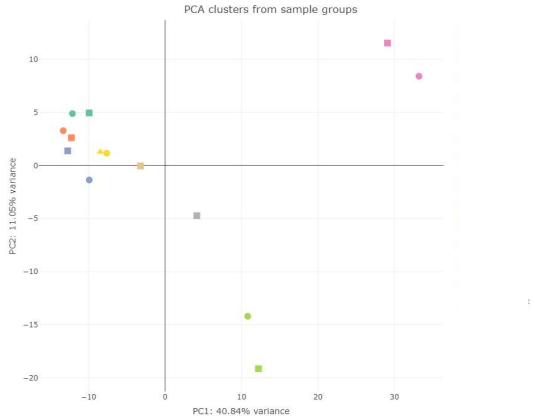
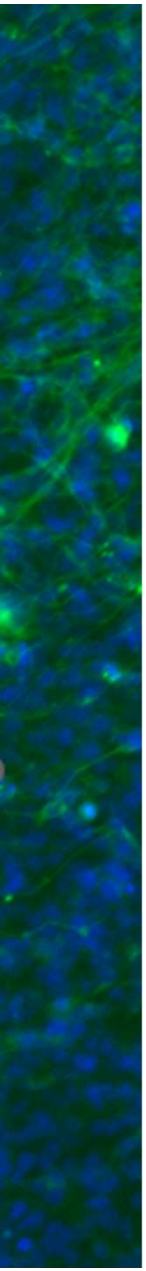




Contact us for more details and tailored solutions



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