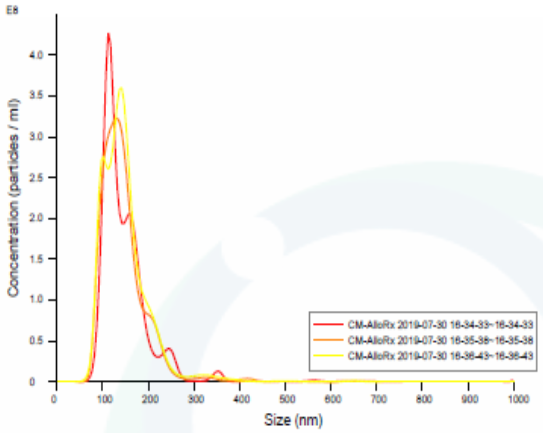
AlloEx vs Competitor:
Exosome Characteristic Comparison

21OCT2019

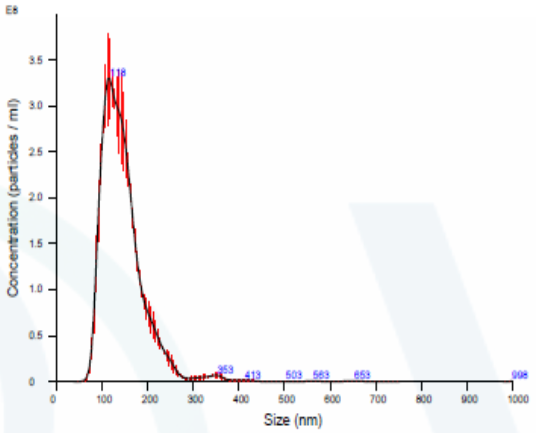
AlloEx Video of Particles Moving Under Brownian Motion From NS300

NANOSIGHT

AlloEx 2019-07-30 16-33-26



FTLA Concentration / Size graph for Experiment:
CM-AlloRx 2019-07-30 16-33-26



Averaged FTLA Concentration / Size for Experiment:
CM-AlloRx 2019-07-30 16-33-26
Error bars indicate + / -1 standard error of the mean

Results

Stats: Merged Data	
Mean:	146.3 nm
Mode:	115.3 nm
SD:	51.1 nm
D10:	98.0 nm
D50:	136.5 nm
D90:	204.4 nm

Stats: Mean +/- Standard Error	
Mean:	146.2 +/- 0.7 nm
Mode:	128.4 +/- 7.7 nm
SD:	51.0 +/- 0.3 nm
D10:	98.1 +/- 1.9 nm
D50:	136.0 +/- 1.9 nm
D90:	204.0 +/- 1.3 nm
Concentration:	5.90e+09 +/- 2.63e+08 particles/ml
	299.3 +/- 13.3 particles/frame
	246.4 +/- 8.6 centres/frame

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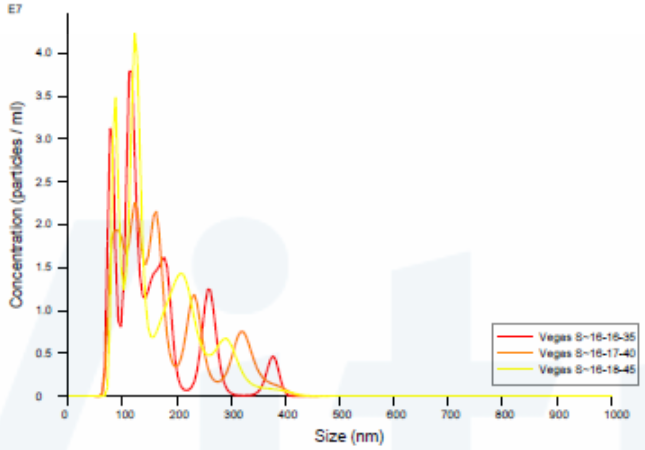
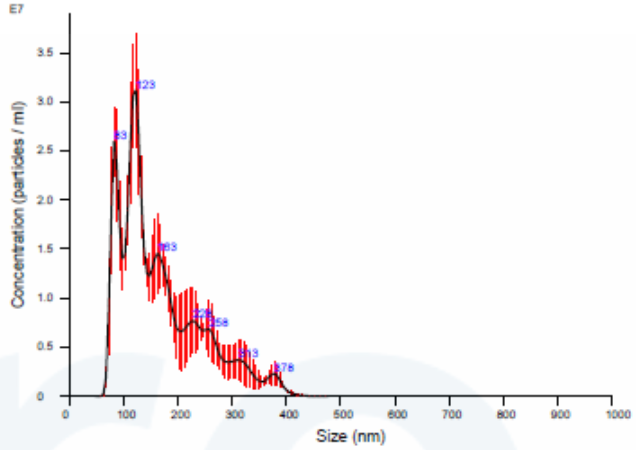
Competitor 2019-07-30 16-12-43



FTLA Concentration / Size graph for Experiment:
Vegas Sample 2019-07-30 16-12-43



Averaged FTLA Concentration / Size for Experiment:
Vegas Sample 2019-07-30 16-12-43
Error bars indicate + / -1 standard error of the mean

Results

Stats: Merged Data	
Mean:	166.9 nm
Mode:	120.5 nm
SD:	76.4 nm
D10:	85.9 nm
D50:	142.8 nm
D90:	281.9 nm

Stats: Mean +/- Standard Error	
Mean:	166.8 +/- 4.9 nm
Mode:	120.8 +/- 2.9 nm
SD:	76.1 +/- 2.4 nm
D10:	85.9 +/- 2.6 nm
D50:	141.6 +/- 5.9 nm
D90:	286.5 +/- 14.7 nm
Concentration:	6.03e+08 +/- 2.17e+07 particles/ml
	30.6 +/- 1.1 particles/frame
	32.5 +/- 1.1 centres/frame

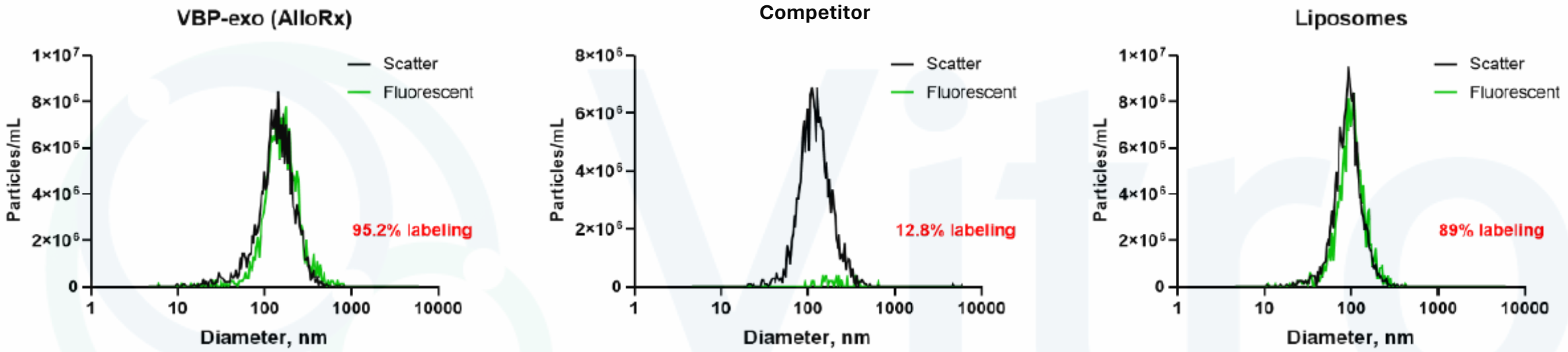
Data Summary of Fluorescent Nano Tracking Analysis via Zetaview

TABLE 1. DATA SUMMARY (fNTA SAMPLE ANALYSIS BY ZETAVIEW)									
#	Dilution	Sample ID	Mode	Median Size (X50) (nm)	X10 (nm)	X90 (nm)	Mean Size, nm	SD (nm)	Original Concentration (particles/mL)
1	250,000	100 nm st_sc	Scatter	98.6	67.6	149.2	108.3	44.2	5e+13
2	100,000	Liposomes_sc	Scatter	90.7	58.0	139.3	97.1	36.3	1.8e+13
3	100,000	Liposomes_fl	Fluorescent	97.9	62.8	153.2	105.6	40.3	1.6e+13
4	1,000	VBP_exo_sc	Scatter	135.0	70.2	232.7	147.2	67.2	2.1e+11
5	1,000	VBP_exo_fl	Fluorescent	152.3	91.7	255.9	171.8	85.1	2.0e+11
6	100	VBP-Comp_sc	Scatter	140.6	81.8	245.5	158.5	73.8	3.9e+9
7	100	VBP-Comp_fl	Fluorescent	201.1	113.9	368.1	235.1	118.7	5.0e+8

Sample ID: Lipsomes= Control
Sample ID: VBP-exo= AlloEx
Sample ID: VBP-Comp= Competitor

Histograms of Purity Overlay

Figure 1:



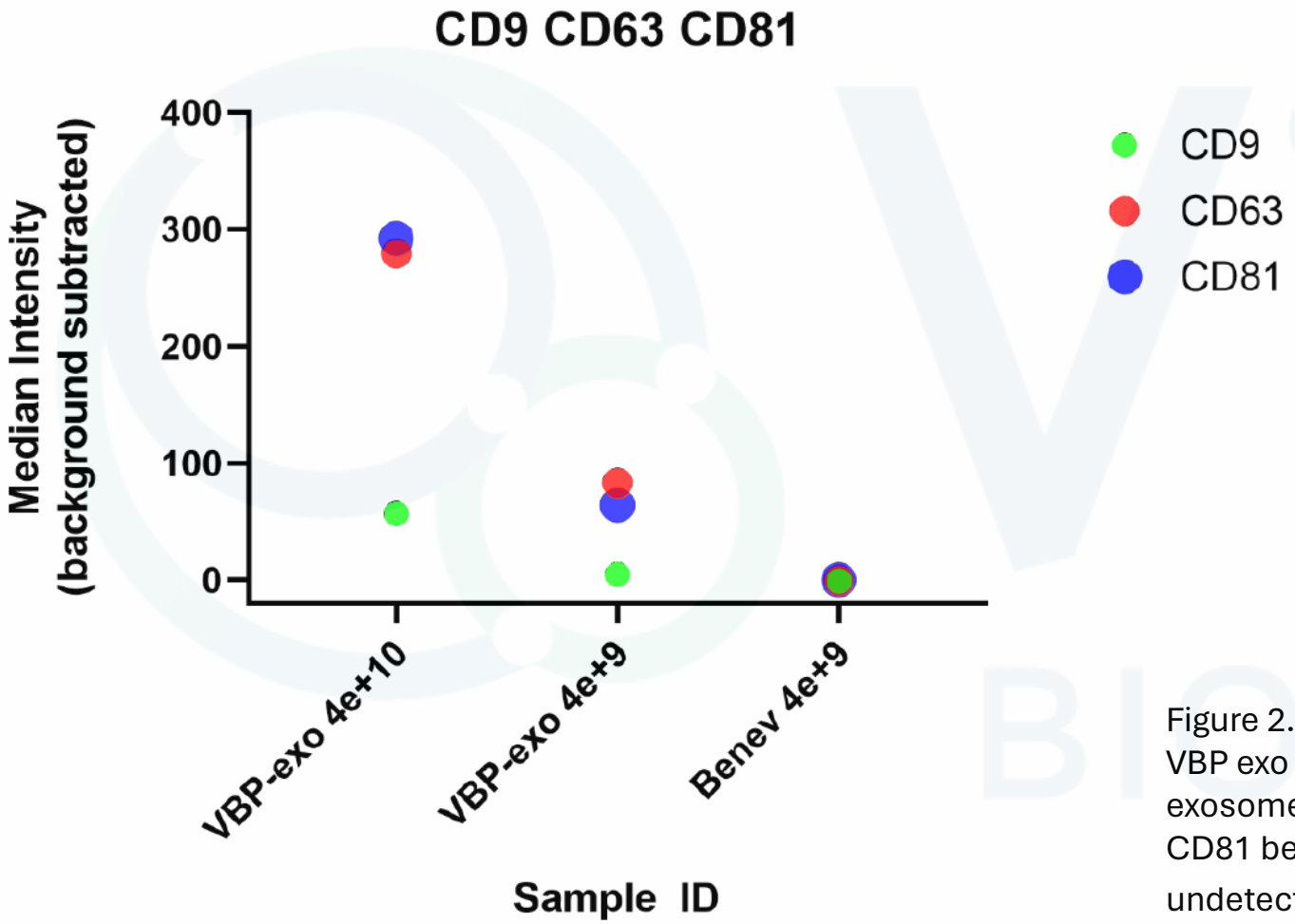
Mode	Scatter	Fluorescent
Sensitivity	70-80	80-85
Shutter	80-100	50
Cycles/positions	2/11	1/11
Frame rate	30	30
Maximum Size	1000	1000
Minimum Size	10	10
Track Length	30-15	15-10
Minimum Brightness	20	20

Figure 1 shows overlay of scatter and fluorescent mode size distribution histograms. Liposomes standard (control) showed 89% labeling, indicating that labeling was successful. It is expected to see over 85% labeling for liposomes standard. fNTA analysis of derived analytes showed that the protocol for extraction and isolation of exosomes from conditioned media is suitable toward samples provided by Vitro Biopharma. The exosomes were over 95% pure according to fNTA data.

The recovery of exosomes from lyophilized powder (Competitor) was less than expected. With assumption that each vial contained about 3B particles, the total particle count was expected to be 60B from 20 vials. After concentration from 20 mL to about 150 µL the expected particle concentration was 8e+12. The achieved concentration was three orders of magnitude lower with only 10% labeling.

Phenotypic Analysis

Figure 2:

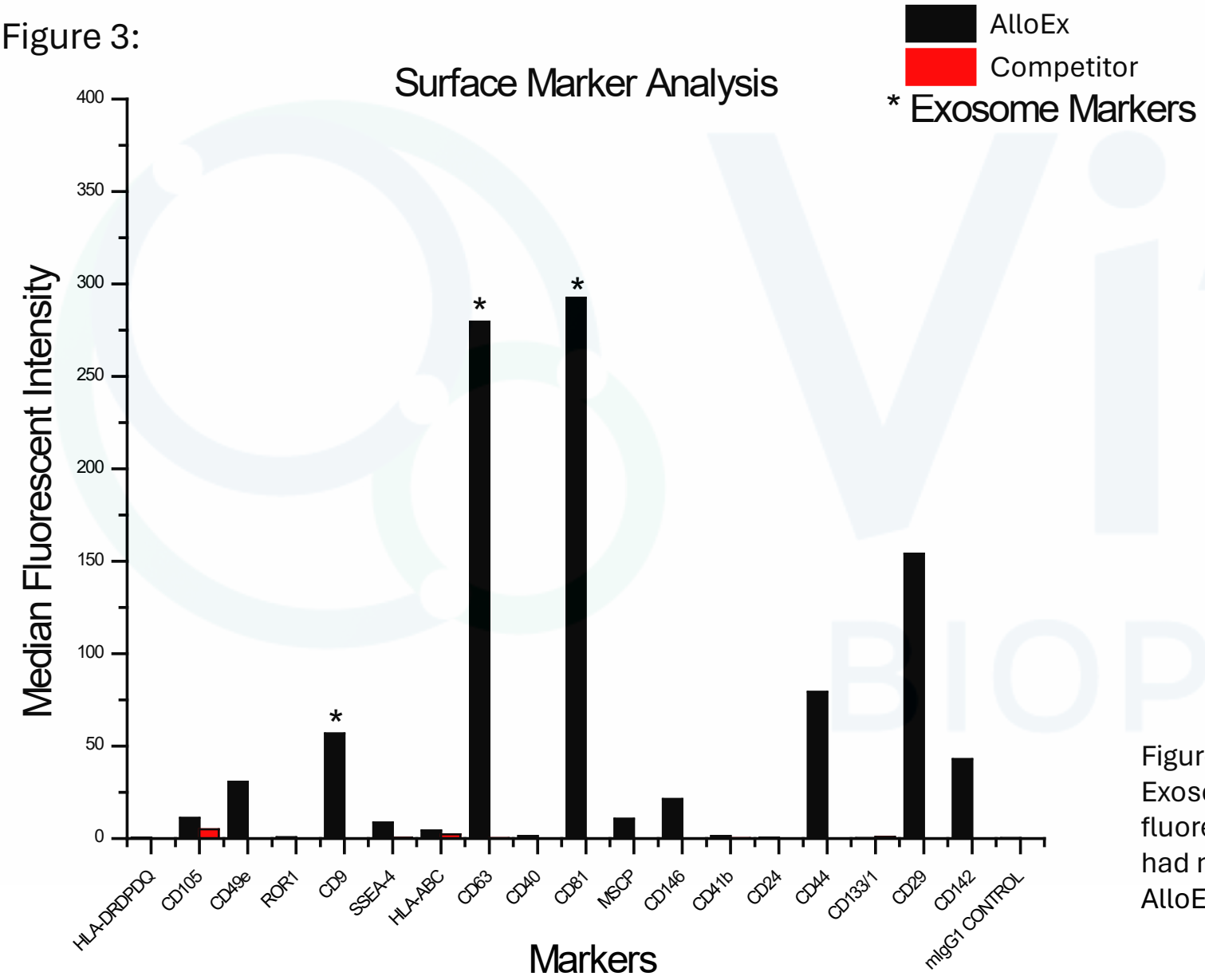


Value	SID	CD9	CD63	CD81
Median Int	VBP-exo 10x	56.82	279.51	292.4
Median Int	VBP-exo 1x	4.58	83.43	64.05
Median Int	VBP-COMP 1x	0.03	0.2	0.03
Median Int	blank	0	0	0

Figure 2. Median Fluorescent intensity of CD9, CD63 and CD81 biomarkers in VBP exo (AlloEx) and Competitor samples of purified exosomes. All three exosome markers were detected in VBP cm (AlloEx) sample with CD63 and CD81 being the most abundant. VBP-COMP CD63, CD81 and CD9 were undetectable.

Phenotypic Analysis- Complete MACSplex Exosome Kit

Figure 3:



- CD105: crucial role in angiogenesis
- CD49e: helps form a receptor for fibronectin
- CD9: modulate cell adhesion and migration and also trigger platelet activation and aggregation
- CD63: play a role in the regulation of cell development, activation, growth, and motility
- CD81: play a role in the regulation of cell development, activation, growth and motility, localized in the tumor-suppressor gene region
- CD146: a matrix molecule that is broadly expressed within the vascular wall
- CD44: involved in cell–cell interactions, cell adhesion and migration
- CD29: involved in cell-cell or cell-matrix adhesion.
- CD142: role in the clotting process is the initiation of thrombin formation

Figure 3 shows the expression from the complete MACSplex Exosome Kit via flow cytometry. AlloEx shows high median fluorescent intensity (MFI) of CD63 and CD81 while Competitor had no expression. All of the phenotypic markers expressed on AlloEx are described above.

Inflammatory Biomarker Analysis

Figure 4:

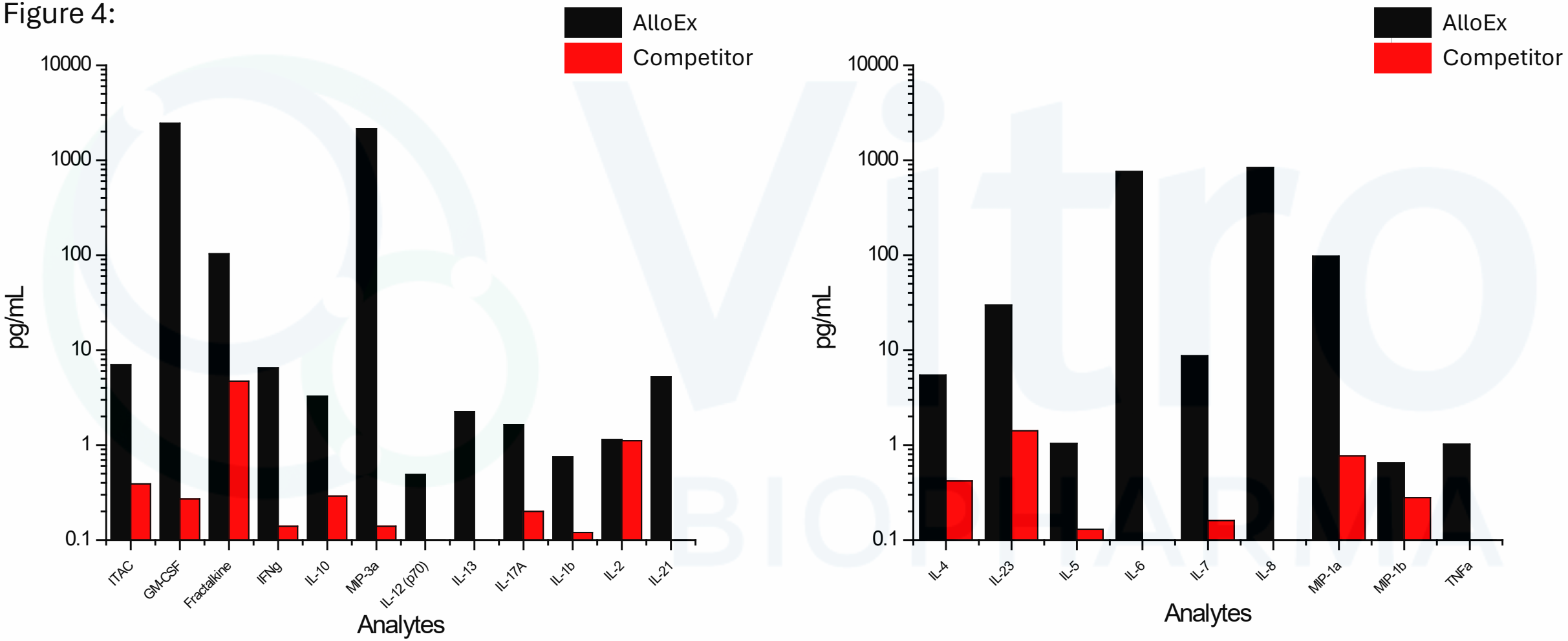


Figure 4. AlloEx and Competitor were ran on the Milliplex Human High Sensitivity T Cell Magnetic Panel to determine pro and anti-inflammatory markers. AlloEx expressed higher levels of both pro and anti-inflammatory markers when compared to Competitor. All inflammatory markers are defined on the next slide.

Inflammatory Biomarker Description

- **Fractalkine/CX3CL**- Anti-Inflammatory
- **GM-CSF**- Pro-Inflammatory
- **IFNγ-** Pro-Inflammatory
- **IL-1β**- Pro-Inflammatory
- **IL-2**- Pro-Inflammatory
- **IL-4**- Anti-Inflammatory
- **IL-5**- Pro-Inflammatory
- **IL-6-** Pro/Anti-Inflammatory
- **IL-7-** Pro-Inflammatory
- **IL-8/CXCL8-** Pro-Inflammatory
- **IL-10**- Anti-Inflammatory
- **IL-12- (p70)** Pro-Inflammatory
- **IL-13**- Anti-Inflammatory
- **IL-17A-** Pro-Inflammatory
- **IL-21-** Pro-Inflammatory
- **IL-23-** Pro-Inflammatory
- **I-TAC/CXCL11-** Pro-Inflammatory
- **MIP-1α/CCL3**- Pro-Inflammatory
- **MIP-1β/CCL4**- Pro-Inflammatory
- **MIP-3α/CCL20**- Pro-Inflammatory
- **TNFα-** Pro-Inflammatory

Verification Data Summary of Fluorescent Nano Tracking Analysis via Zetaview

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#	Dilution	Sample ID	Mode	Median Size (X50) (nm)	X10 (nm)	X90 (nm)	Mean Size, nm	SD (nm)	Original Concentration (particles/mL)
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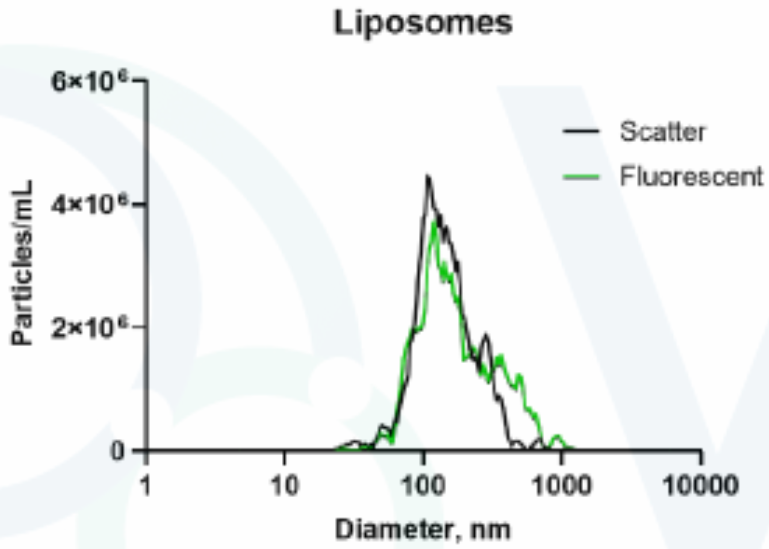
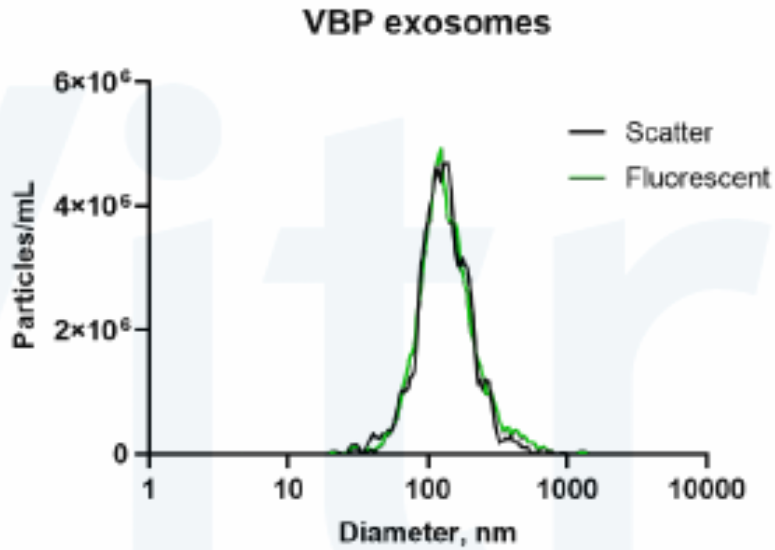
2	20,000	Liposomes_sc	Scatter	133.7	80.9+	278.8	161.3	90.3	2.4e+12
3	20,000	Liposomes_fl	Fluorescent	150.6	80.3	440.3	215.3	162.5	2.4e+12
4	1,000	VBP_exo_sc	Scatter	127.9	75.8	217.4	144.3	77.5	8.6e+10
5	1,000	VBP_exo_fl	Fluorescent	127.5	77.5	245.2	154.3	90.8	8.6e+10

Sample ID: Lipsomes= Control
Sample ID: VBP-exo= AlloEx

Histograms of Purity Overlay





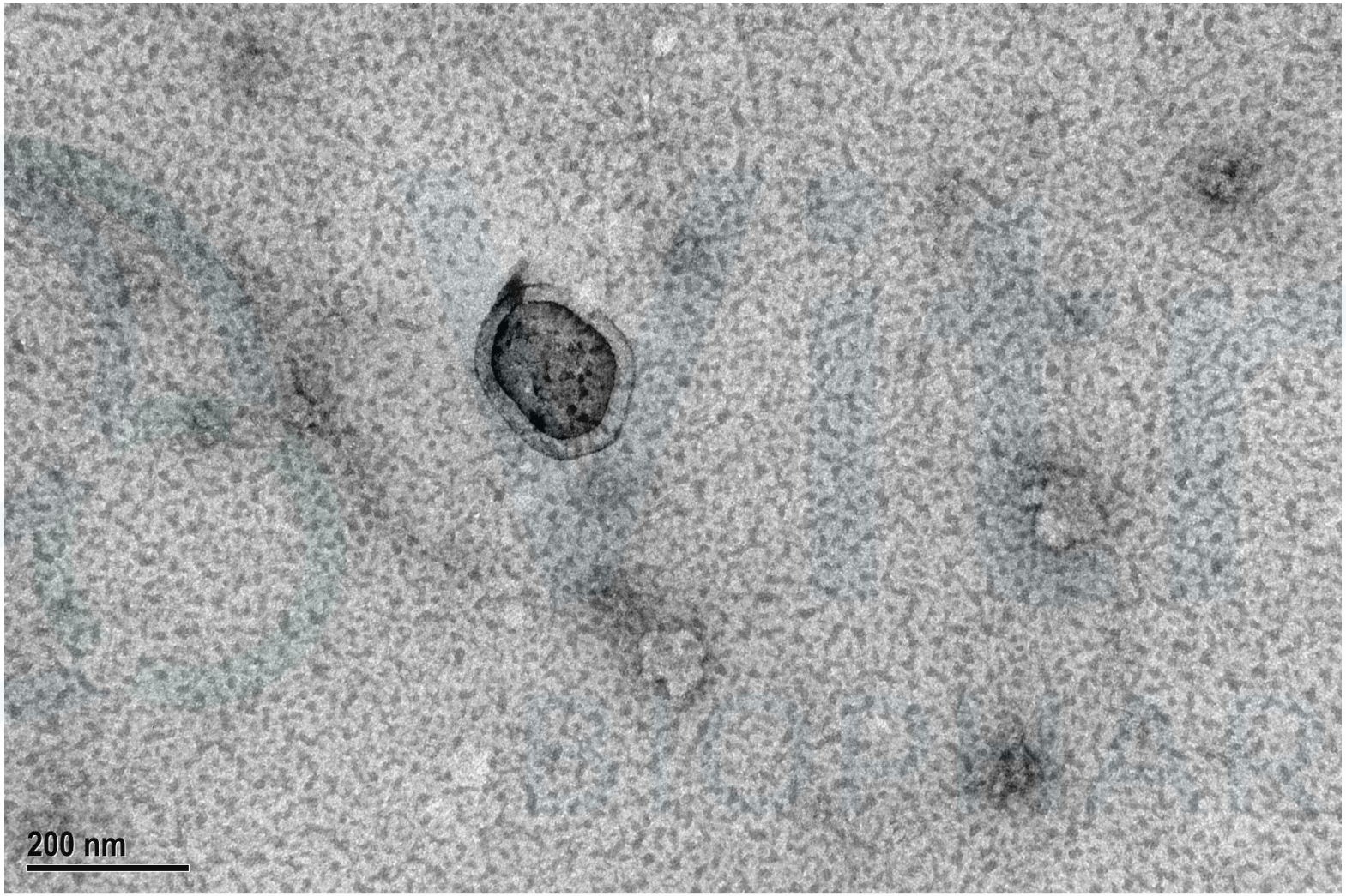
Mode	Scatter	Fluorescent
Sensitivity	70-80	80-85
Shutter	80-100	50
Cycles/positions	2/11	1/11
Frame rate	30	30
Maximum Size	1000	1000
Minimum Size	10	10
Track Length	30-15	15-10

Figure 1 shows overlay of scatter and fluorescent mode size distribution histograms. All samples showed high percent of fluorescent labeling suggesting high purity of exosomes. Liposomes standard showed 89% labeling, indicating that labeling was successful. It is expected to see over 85% labeling for liposomes standard.

Figure 1. Overlay of particle size distribution histograms recorded in scatter and fluorescent modes for liposome labeling standard and VBP exosomes.

fNTA analysis of derived analytes showed that the protocol for extraction and isolation of exosomes from conditioned media is suitable toward samples provided by Vitro Biopharma. The exosomes showed 100% labeling according to fNTA data.

AlloEx Transmission Electron Microscope (TEM)



Name: VBP CCMexo-0002 Indicated magnification: 120kx Operator: DF Acquisition date: 10/16/2019