

1. SEC-based method

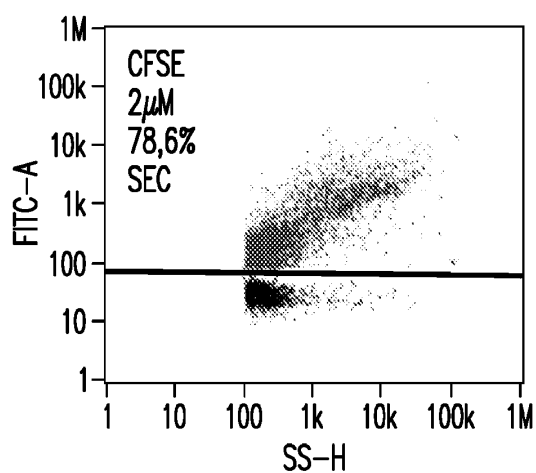


FIG. 1A

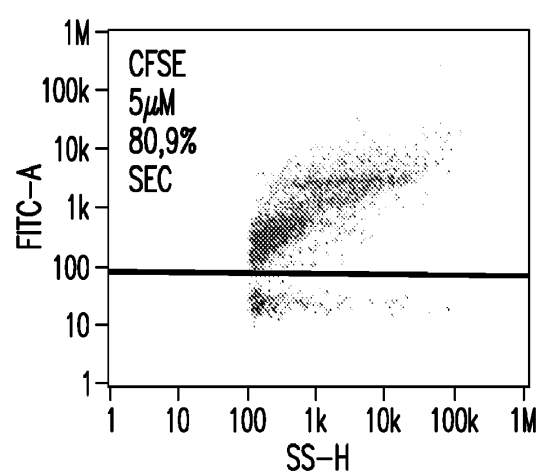


FIG. 1B

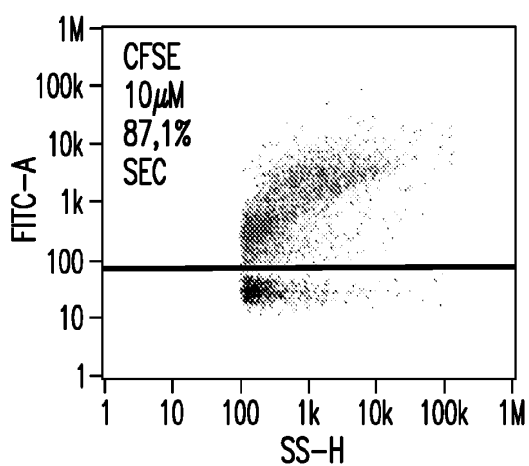


FIG. 1C

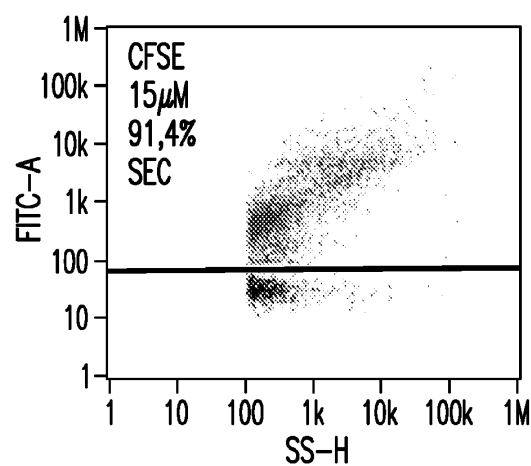
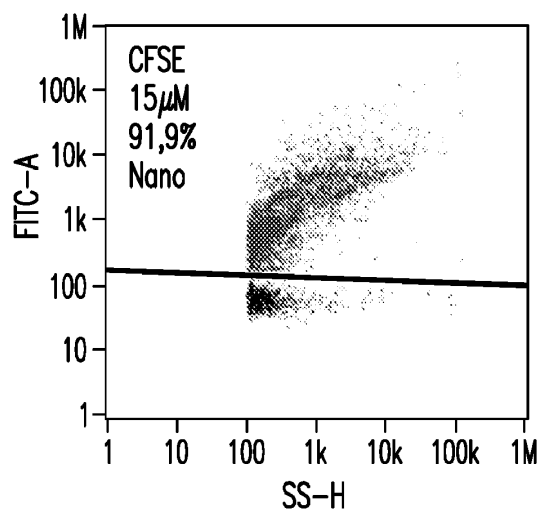
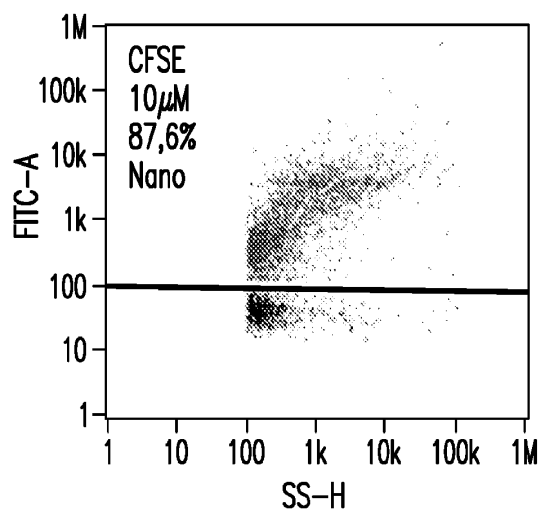
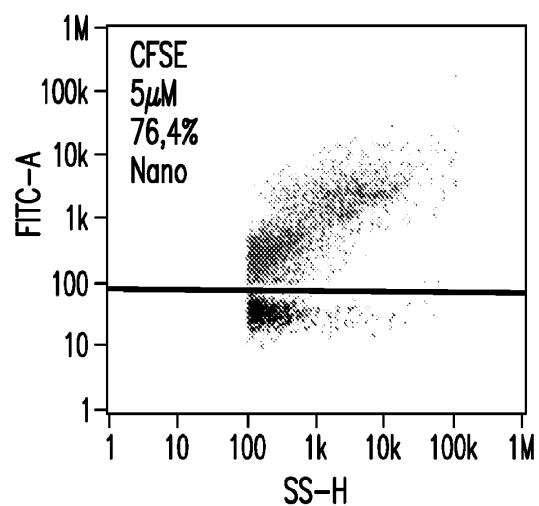
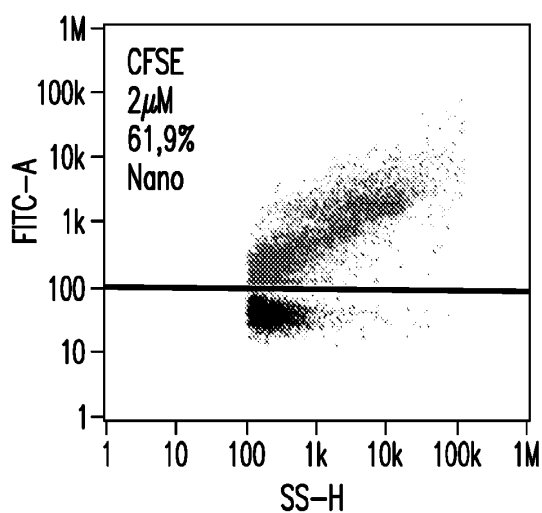


FIG. 1D

2. Filtration-based method



		Input						Fluorescence: trigger channel SS					
ul after Nanosep	Sample (wash with 3ml)	Top part	threshold SS	threshold FITC	threshold PE	median nm	Mean nm	%labelling	threshold SS	threshold FITC	threshold PE	median nm	Mean nm
Conc supernatants	89	Nanosep 300	2,01E+08	21,4	95,7	68,25	71,83	91,6%	98,1	35,9	98,6	65,25	69,78
	54	Nanosep 100						97,7%	99,6	56,6	102,8	65,25	71,55
	64	Amicon 100						42,0%	101,2	435,3	161,4	78,75	82,19
	83	Amicon 50						31,8%	102	438,2	161,1	85,75	95,83
purified EVs	60	Nanosep 300	1,82E+08	17,7	93,7	71,75	75,46	96,4%	95,7	33,1	97,9	64,65	74,28
	108	Nanosep 100						93,6%	97,5	20,6	95,4	73,75	77,97
	92	Amicon 100						79,9%	97,7	145,8	116,3	71,25	75,23
	113	Amicon 50						79,3%	100,3	152,9	117,7	70,75	74,76

FIG. 2

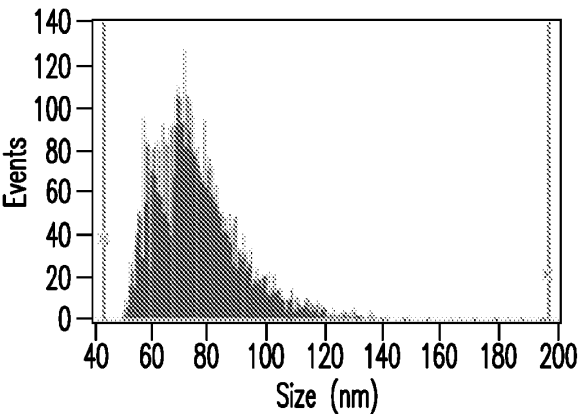
SN: FNAU30T19101043
Software: V1.08
Operator: NF
Sample Pressure: 1.0 Kpa

Laser: 15/50 mW 488 @
SS Decay: 10%
Threshold/sub: 93.7 20.5 2.6 NaN/0 0 0
Min Width: 03 ms

Size Information

Gating Range 43.35 – 196.65 nm

Total Events	5864
Gating Events	5864
% of all	100.00
Median	73.25 nm
Mean	75.65 nm
Std Dev.	15.06 nm



Concentration Information

	Particle Number	Dilution Factor
STD	5752	200
Blank	168	—
Sample	6032	1

STD Concentration 4.12E+10 Particles/mL
Sample Flow Rate 27.92 nL/min
Sample Concentration 2.10E+8 Particles/mL
Corrected Ratio: 5864/5864 100.0%

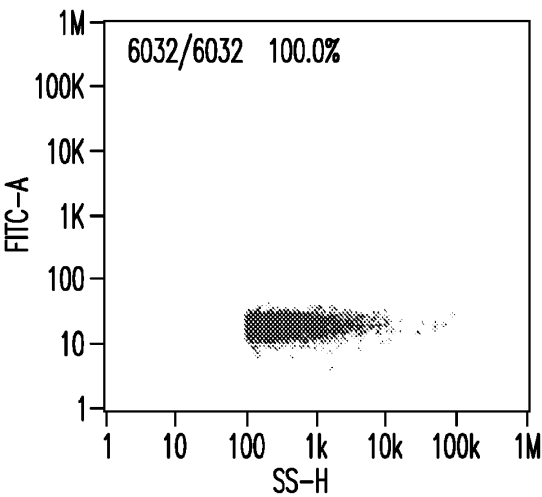


FIG. 3A

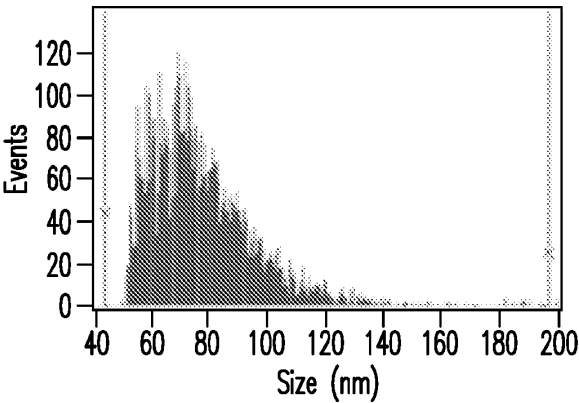
SN: FNAU30T19101043
Software: V1.08
Operator: NF
Sample Pressure: 1.0 Kpa

Laser: 15/50 mW 488 @ 15/100 mW 640 @
SS Decay: 10%
Threshold/sub: 92 20.7 9 NaN/0 0 0 0
Min Width: 03 ms

Size Information

Gating Range 43.35 - 196.65 nm

Total Events 5682
Gating Events 5651
% of all 99.45
Median 73.25 nm
Mean 77.23 nm
Std Dev. 18.70 nm



Concentration Information

	Particle Number	Dilution Factor
STD	5752	200
Blank	178	—
Sample	5960	4

STD Concentration 4.12E+10 Particles/mL
Sample Flow Rate 27.92 nL/min
Sample Concentration 8.14E+8 Particles/mL
Corrected Ratio: 5682/5682 100.0%

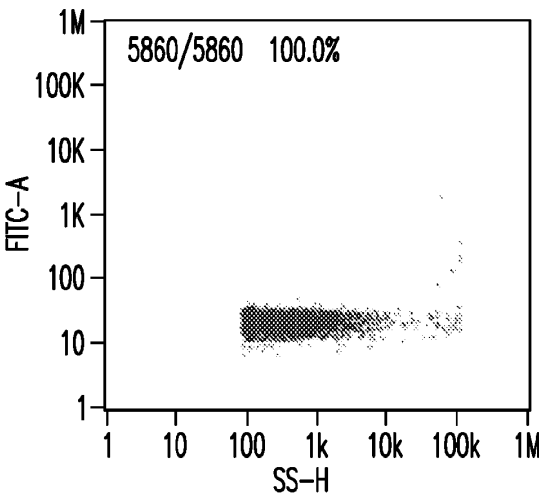


FIG. 3B

CD9 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
Nanosep 300k	33,03%	2,37%	71,92	0,58	76,32	0,55
SEC	34,47%	1,31%	72,08	4,48	75,56	4,20

CD63 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
Nanosep 300k	12,33%	0,55%	72,58	2,31	78,72	2,09
SEC	10,80%	1,48%	73,25	2,78	81,71	3,41

CD81 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
Nanosep 300k	45,00%	2,13%	68,92	0,29	73,62	0,44
SEC	46,63%	4,67%	70,92	1,61	75,08	0,93

FIG. 4A

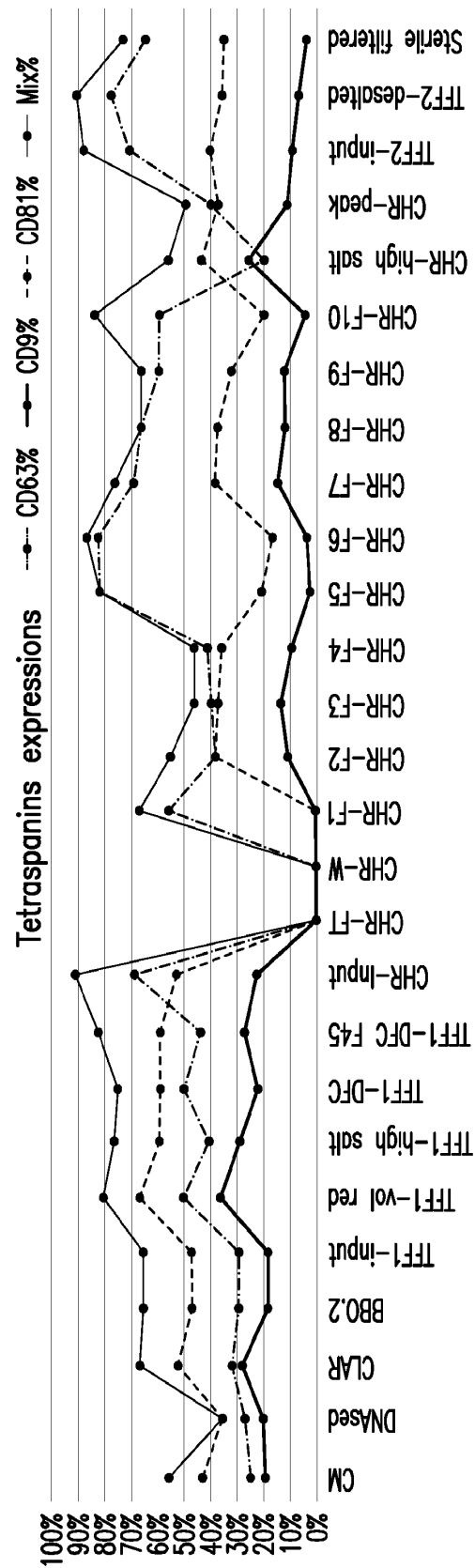


FIG. 4B

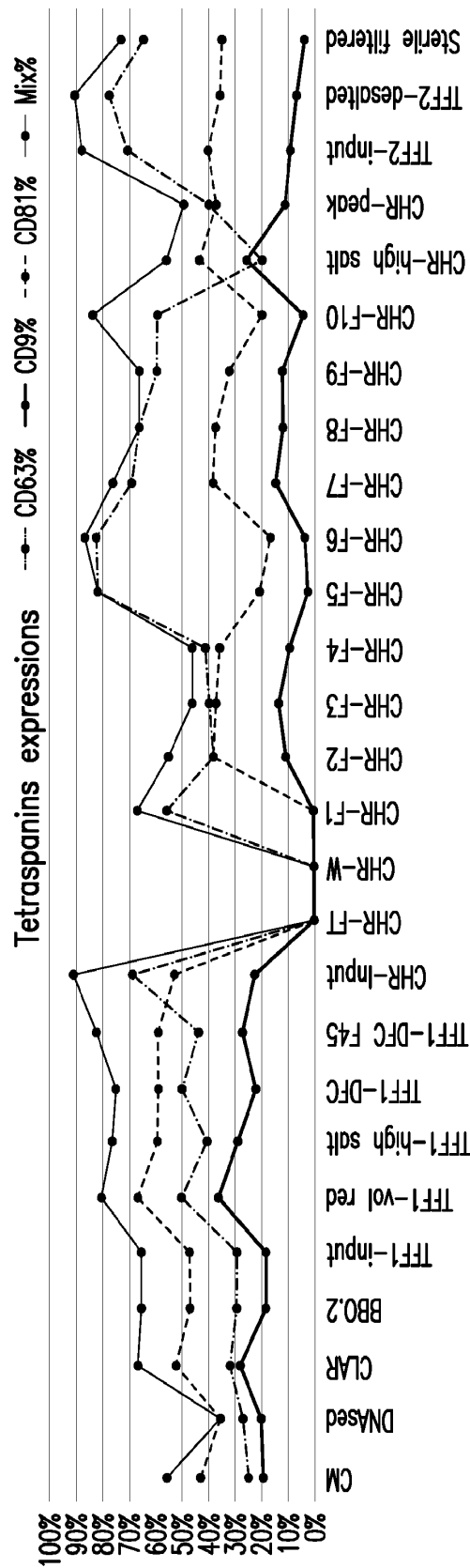


FIG. 4C

CD9-positive events

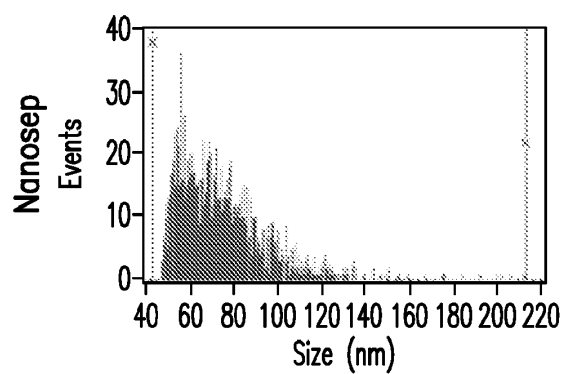


FIG. 5A

CD63-positive events

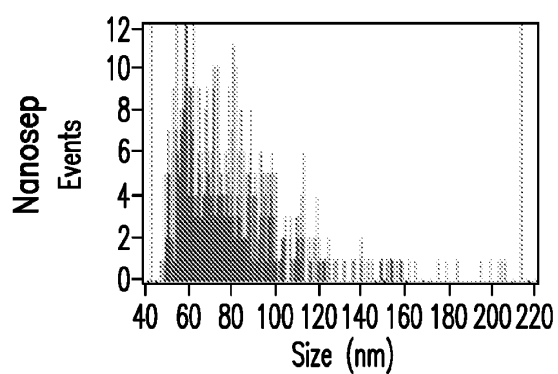


FIG. 5B

CD81-positive events

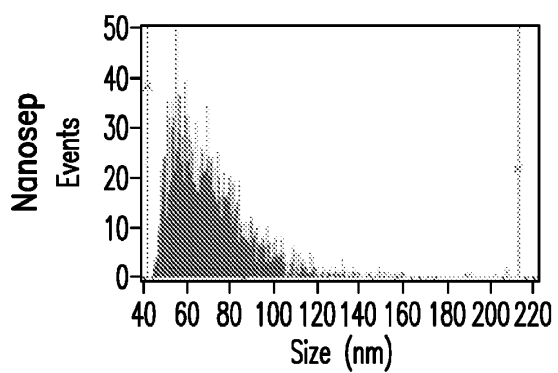


FIG. 5C

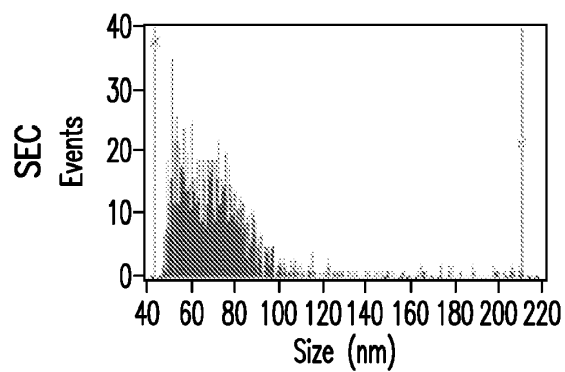


FIG. 5D

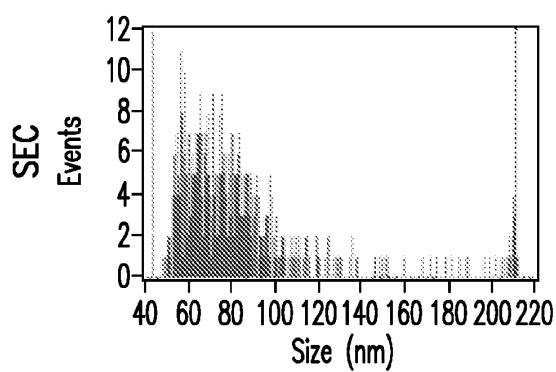


FIG. 5E

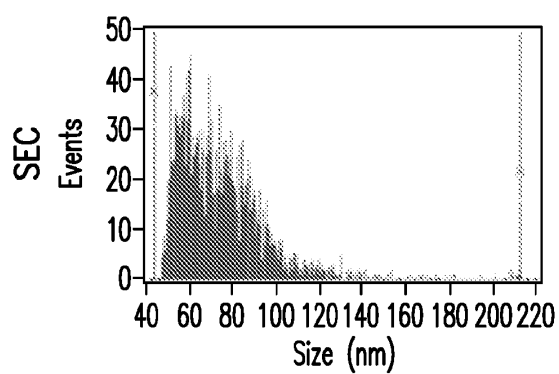


FIG. 5F

PE (Exbio) Antibodies	dilution	threshold		Events		Trigger PE under SS
		SS	PE	Positive	Total	
CD9	PBS	92,2	115,1	1	468	0
	100	90,4	156,4	8	549	205
	300	91,6	125	5	482	137
	500	91,6	122,1	4	481	106
	PBS	94	116	0	1402	0
CD63	100	92,1	138,5	5	1122	163
	300	90,7	119,8	5	1158	55
	500	93,2	119,5	6	1365	62
	PBS	92,6	117,5	0	847	0
	100	90,7	166,3	9	772	247
CD81	300	93	126,5	2	821	153
	500	93,2	122,2	1	809	77

FIG. 6A

AF4888 (R&D System) antibodies	dilution	threshold		Events		Trigger FITC under SS
		SS	FITC	Positive	Total	
CD81	PBS	93,8	21,2	1	864	0
	100	92,6	53	111	757	7106
	200	92,7	27,3	143	723	8465
	300	92,8	25,9	96	842	5318
	500	92,6	25,3	33	773	1901
	1000	93	24,8	24	754	1751
CD63	PBS	90,3	21	1	468	0
	300	97,6	49,3	36	331	4286
	500	93,4	42,4	15	333	2098
	1000	94,3	24,4	43	368	5557
	2000	96,9	21,4	14	367	2157

FIG. 6B

CD9 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
dilution 1:1000	35,47%	4,53%	65,92	1,15	70,26	1,63
SEC	34,47%	1,31%	72,08	4,48	75,56	4,20

CD63 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
dilution 1:1000	14,97%	2,39%	68,75	0,50	75,20	1,89
SEC	10,80%	1,48%	73,25	2,78	81,71	3,41

CD81 PE	% labelling		median nm		mean nm	
	Average (n=3)	st dev	Average (n=3)	st dev	Average (n=3)	st dev
dilution 1:1000	43,20%	4,06%	71,25	1,32	74,73	0,78
SEC	46,63%	4,67%	70,92	1,61	75,08	0,93

FIG. 7A

MSC 150x CM	%s staining	threshold		Median nm	Mean nm	PE trigger	Total events	
		SS	PE				Positive	Total
unstained	–	157,4	123,1	56,25	61,52	–	–	3629
CD63	26,3%	155,7	127,5	58,75	63,35	1170	919	3796
CD81	53,6%	148,4	126,4	59,75	64,29	2602	2185	4394
MIX CD63–81	65,2%	136,2	136,2	56,75	61,64	4125	3021	4984

Results from MSC 150x conditioned media: CD63, CD81, MIX of CD63 and CD81

FIG. 7B

HEK 30x CM	% staining		threshold		Median nm		Mean nm		PE trigger		Total Events	
	average	CV	average	st dev	average	st dev	average	st dev	average	st dev	average	st dev
unstained	0,00%	0%	121,17	0,3	52,92	1,0	61,02	0,6	0	0	2576	128
CD9	42,73%	4%	139,00	4,4	52,58	1,2	60,97	1,1	497	148,7	3504	145
CD63	26,60%	5%	129,13	1,2	54,58	1,0	64,83	0,2	295	26,4	2581	166
CD81	63,57%	0%	123,97	1,2	52,75	0,5	58,71	0,6	1073	109,8	3641	640
MIX CD9-63-81	87,89%	6%	143,07	4,4	51,75	0,9	56,99	0,7	717	69,2	2305	418
Isotype	0,57%	27%	157,13	3,2	68,75	15,1	92,04	10,3	162	16,7	2408	168

Results from HEK 30x conditioned media: CD9, CD63, CD81, isotype and MIX of CD9, CD63, CD81

FIG. 7C

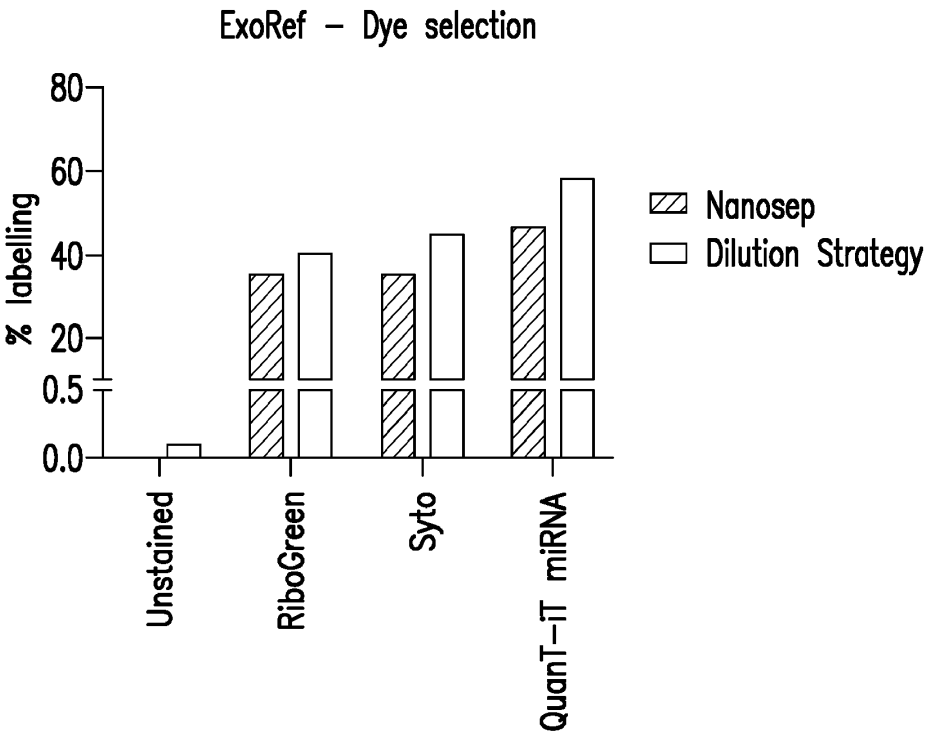


FIG. 8A

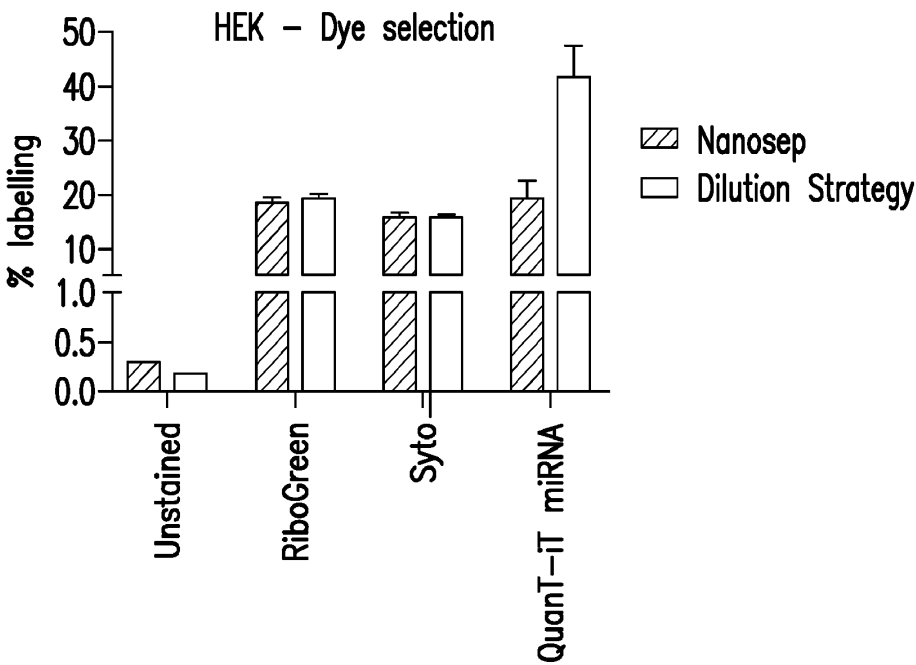


FIG. 8B

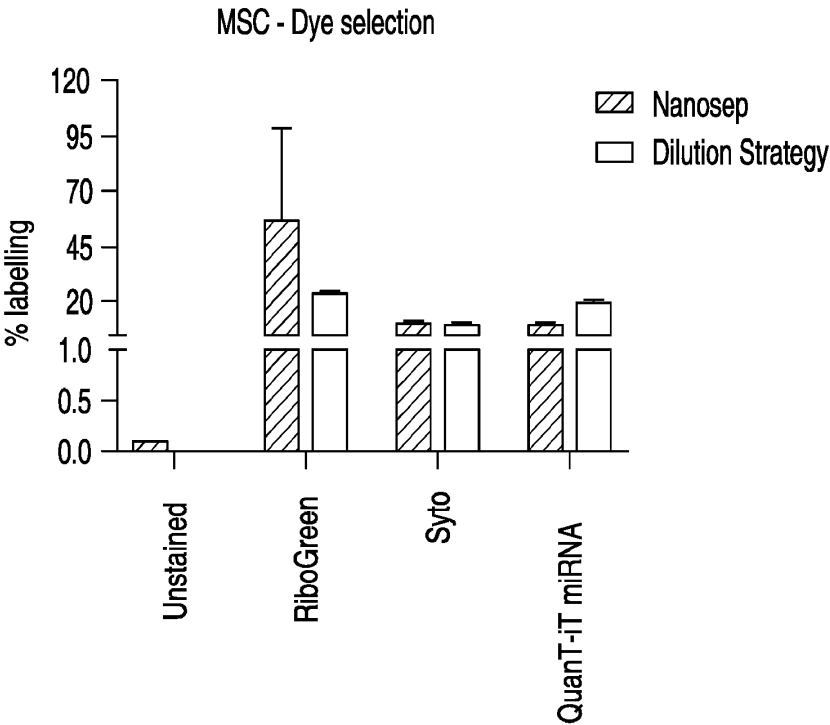


FIG. 8C