

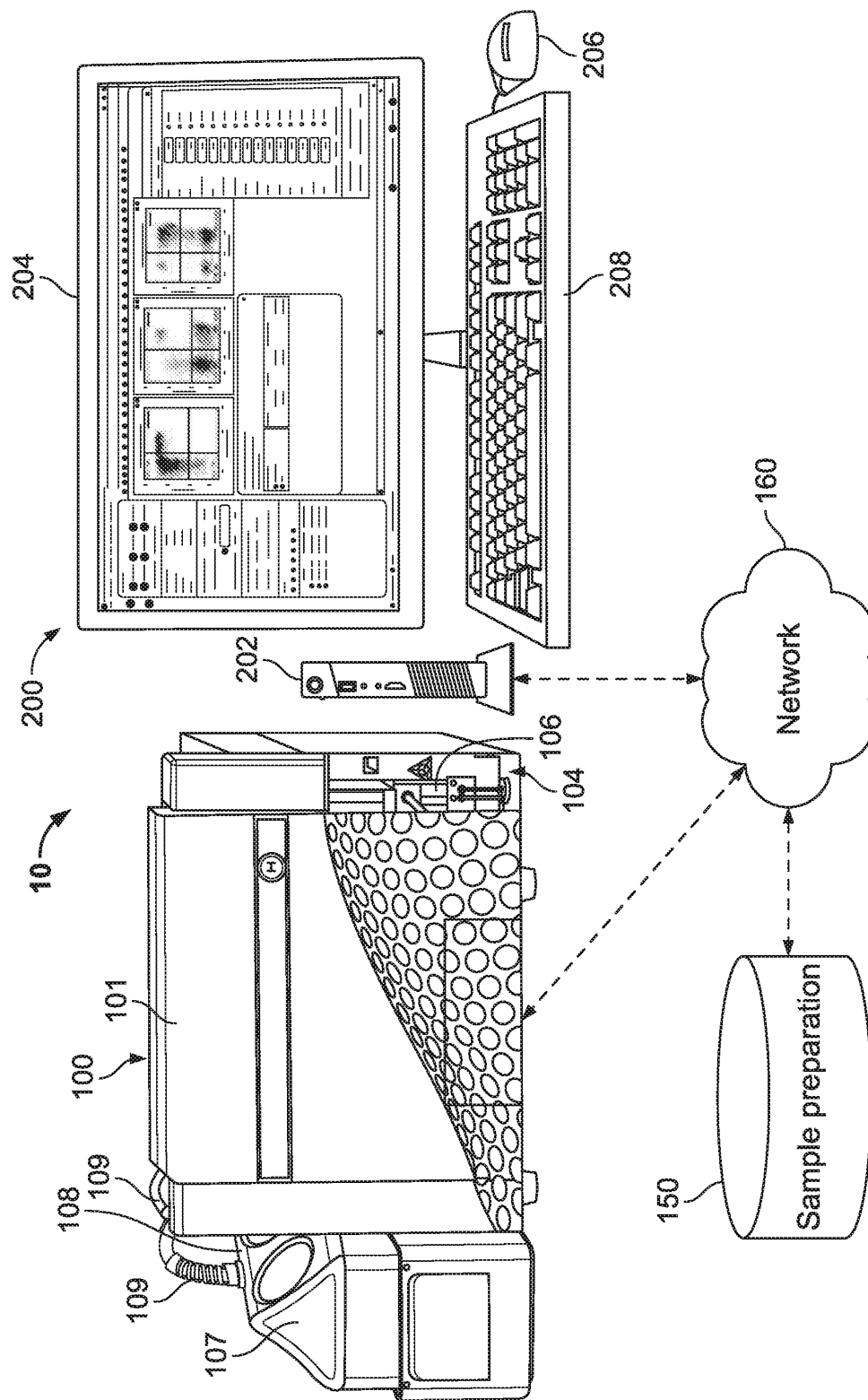
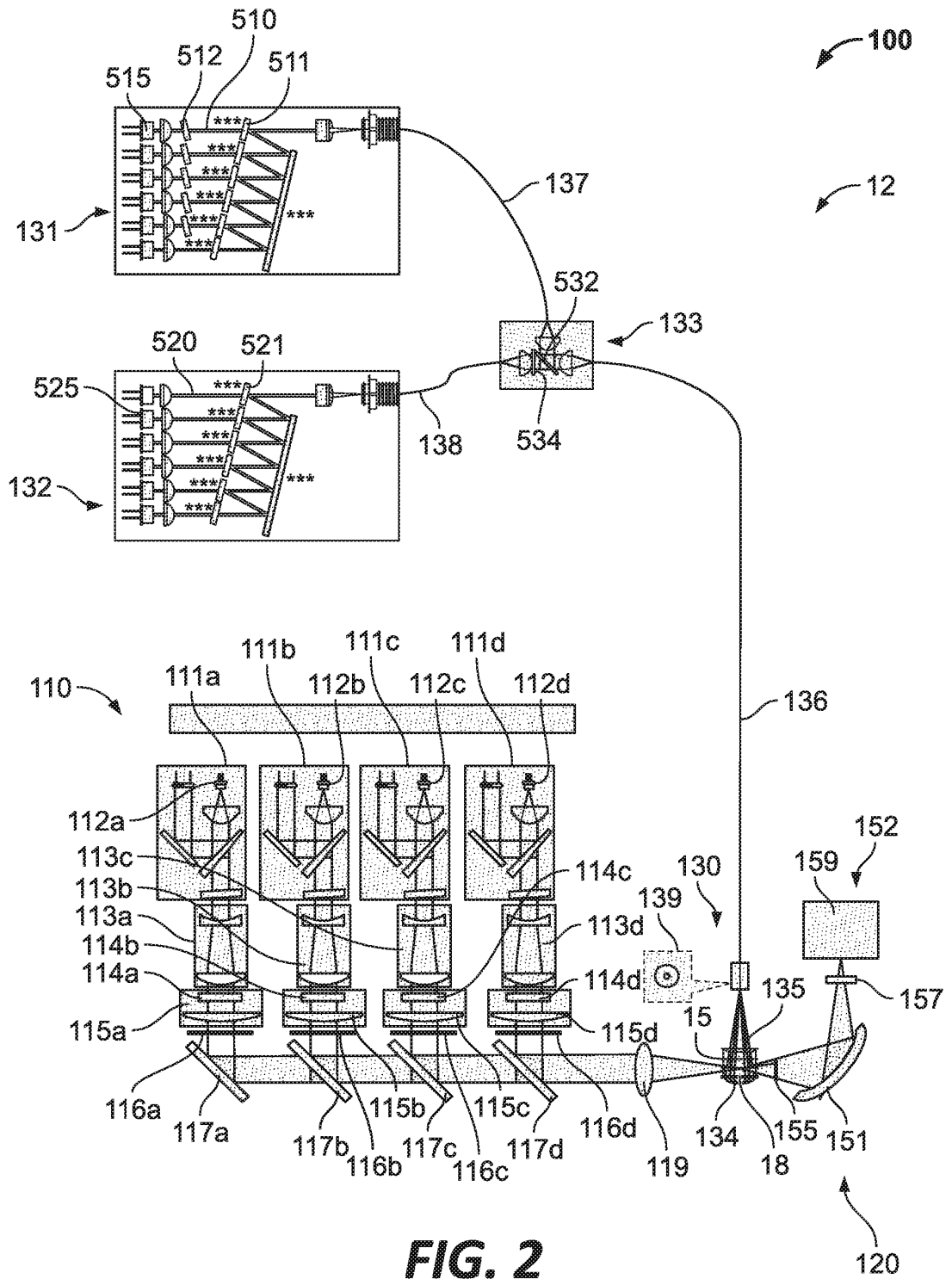
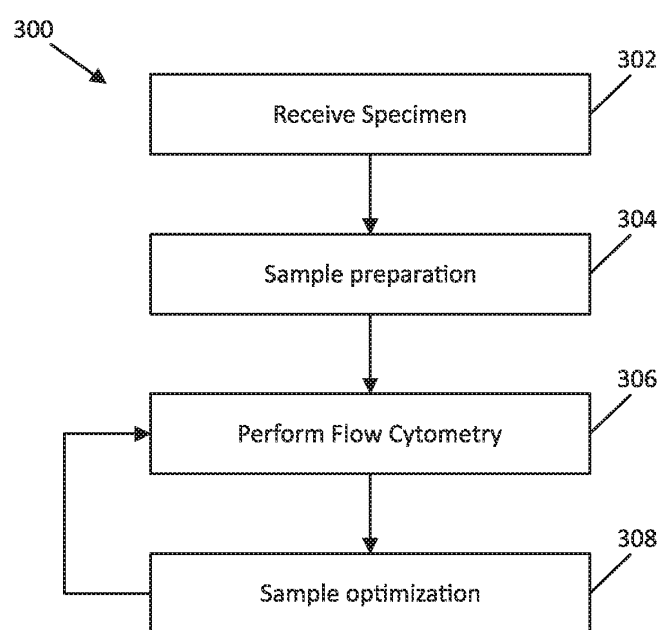
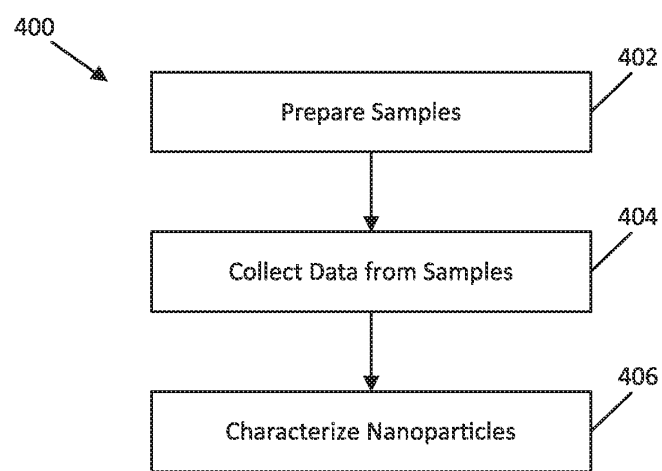


FIG. 1

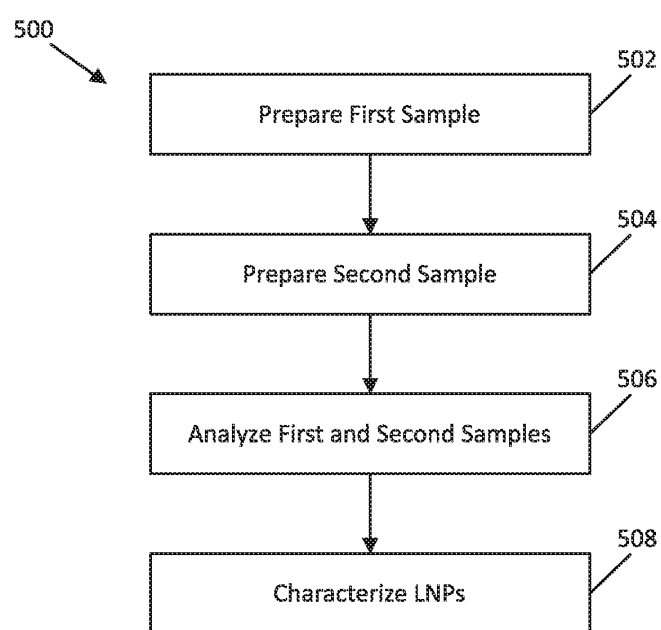


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**FIG. 3**

**FIG. 4**

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**FIG. 5**

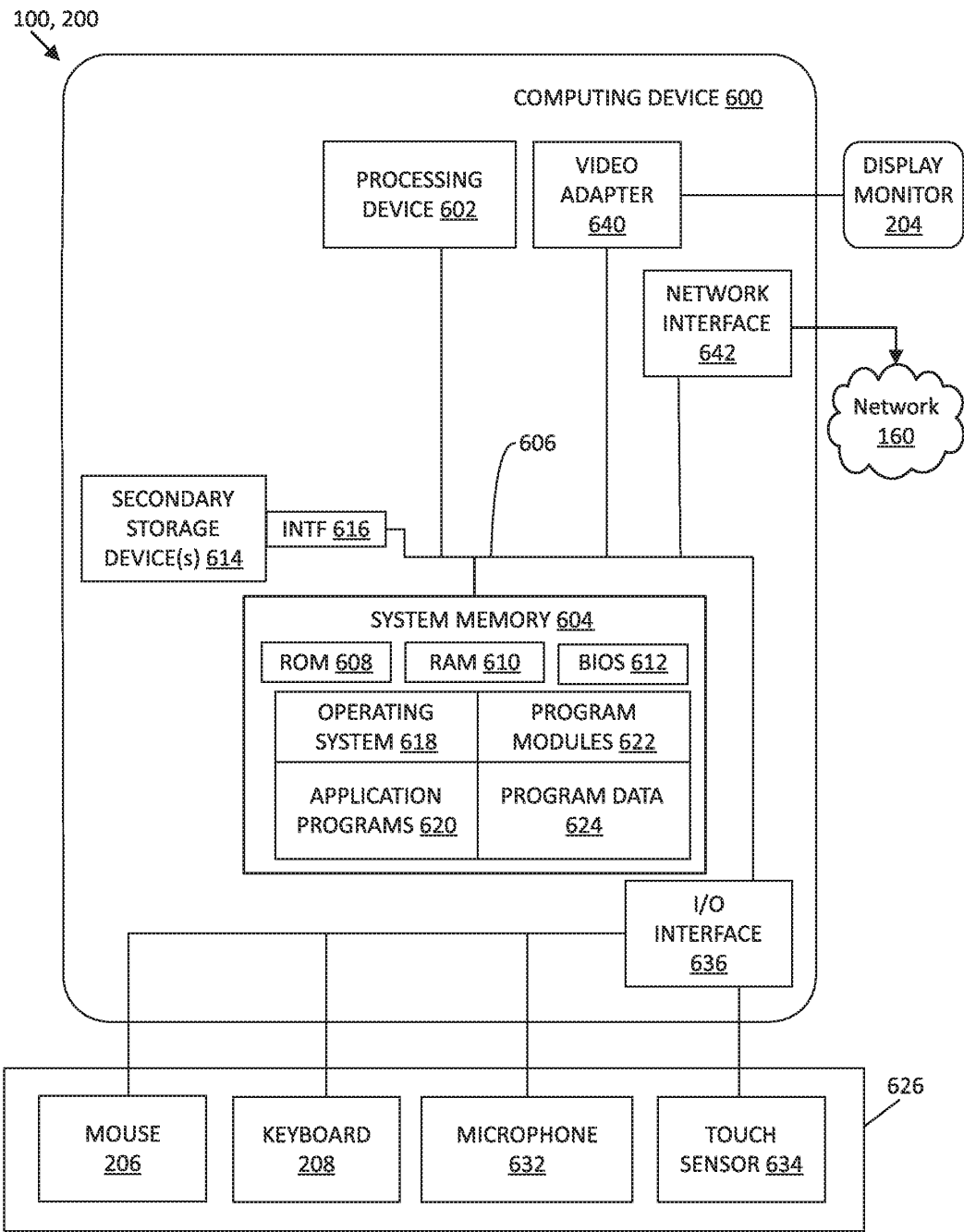


FIG. 6

LNP-LNP1-Raw data

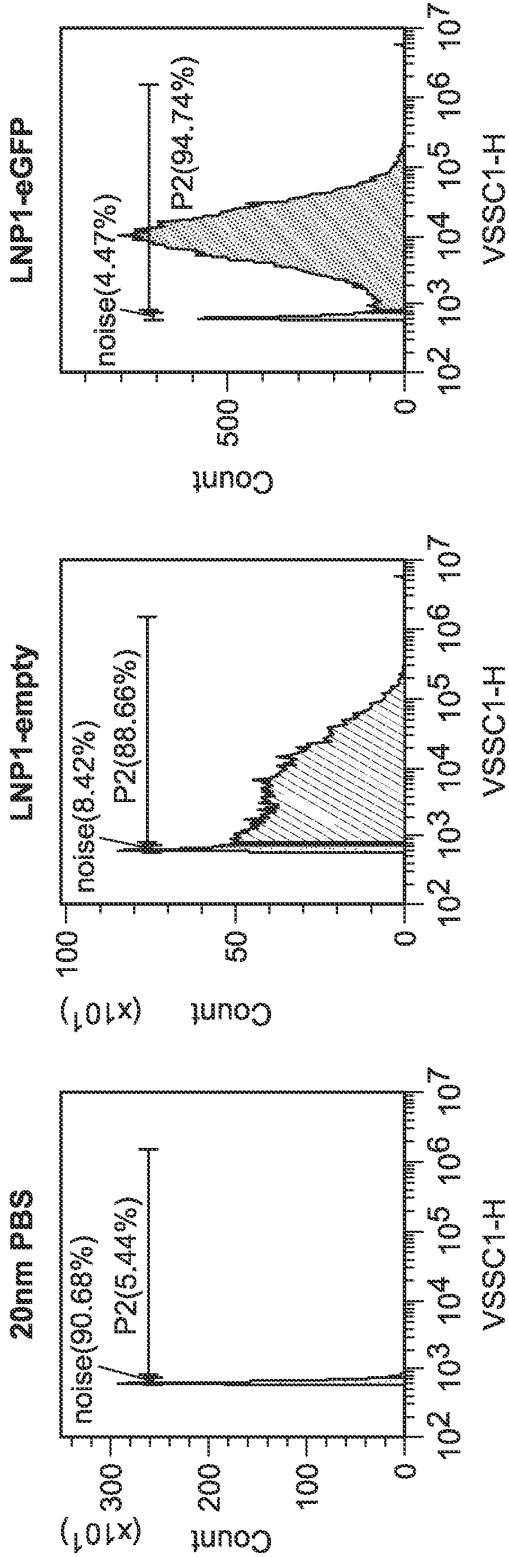


FIG. 7

LNPs-RAW data

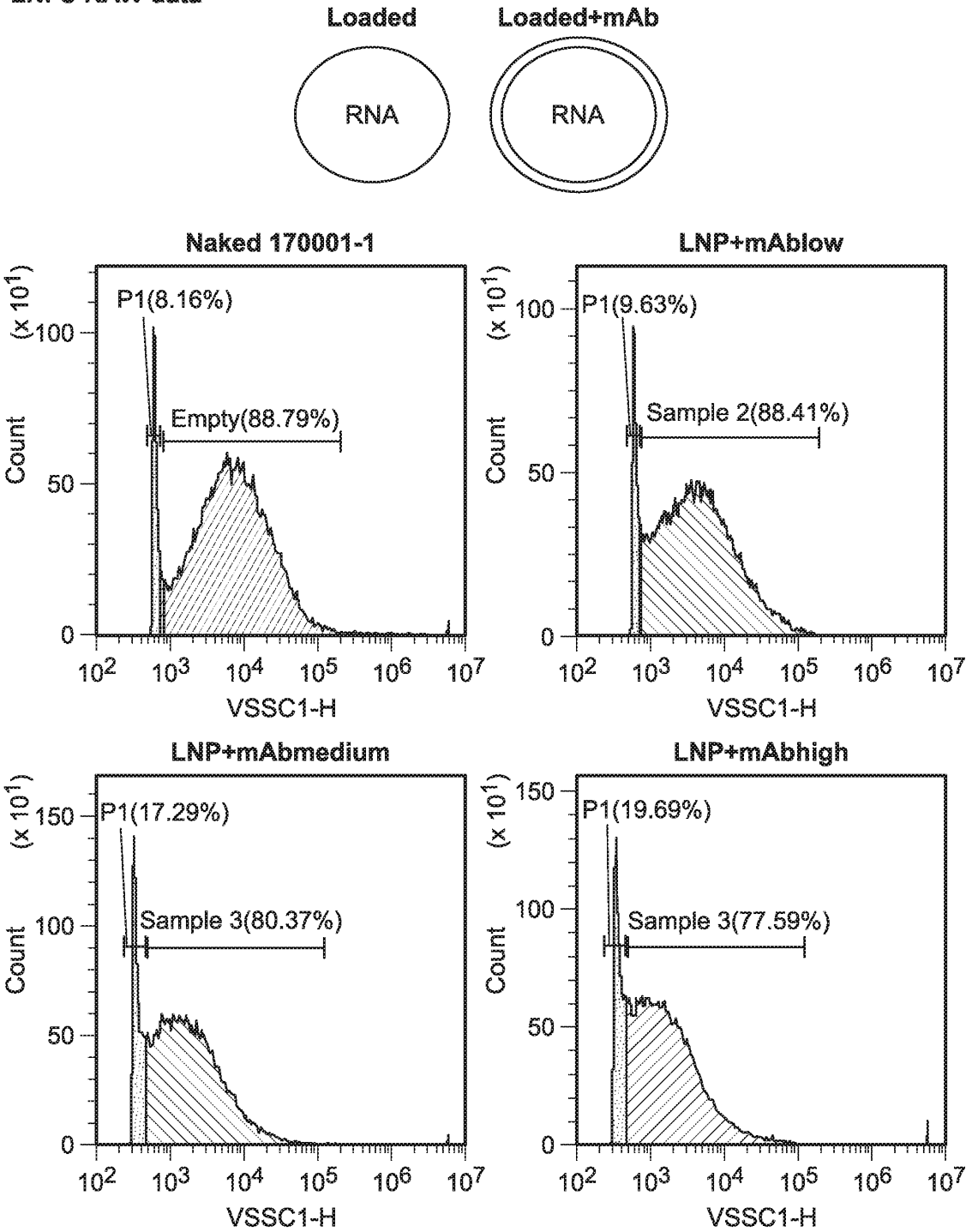
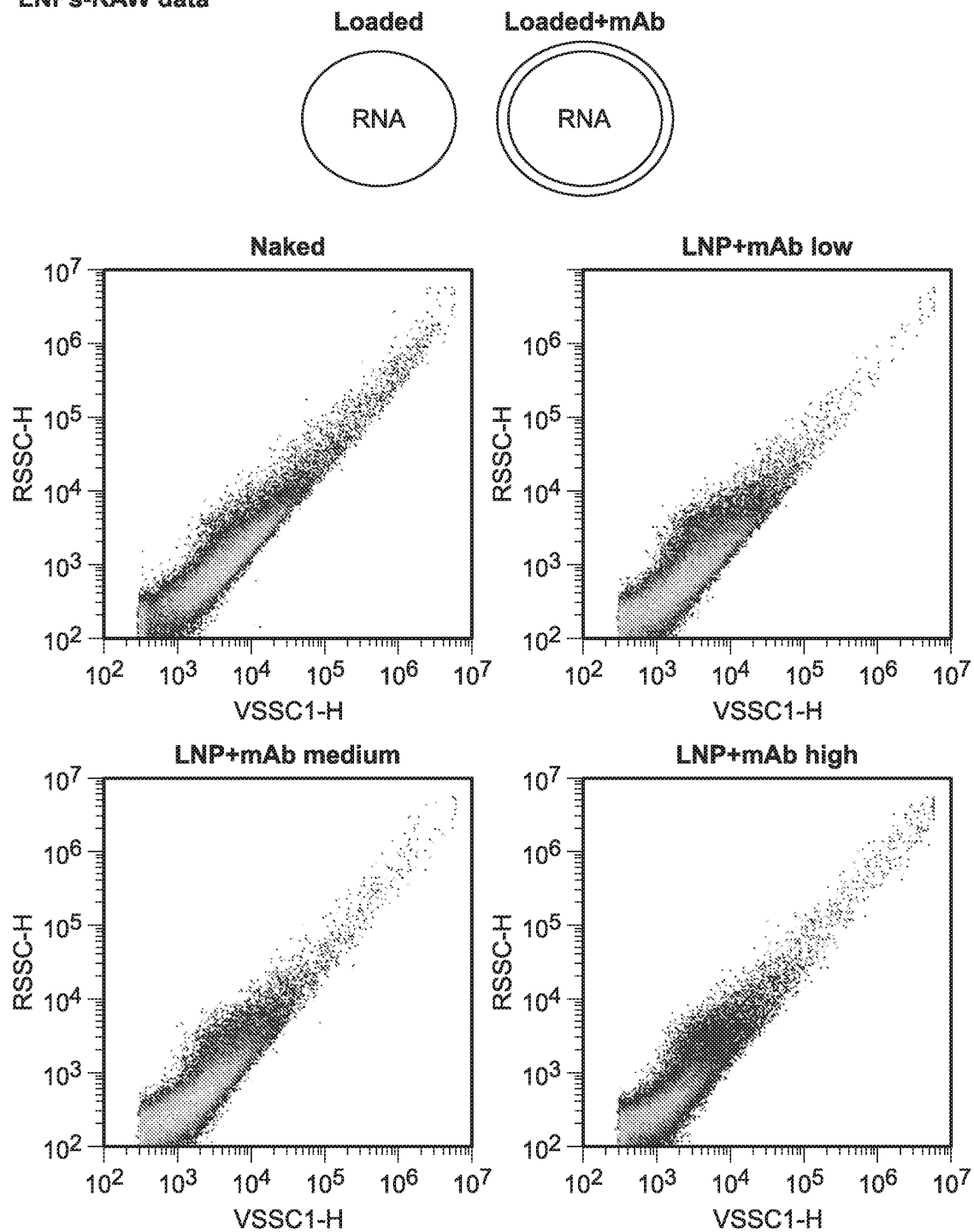


FIG. 8A

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LNPs-RAW data

**FIG. 8B**

SUBSTITUTE SHEET (RULE 26)

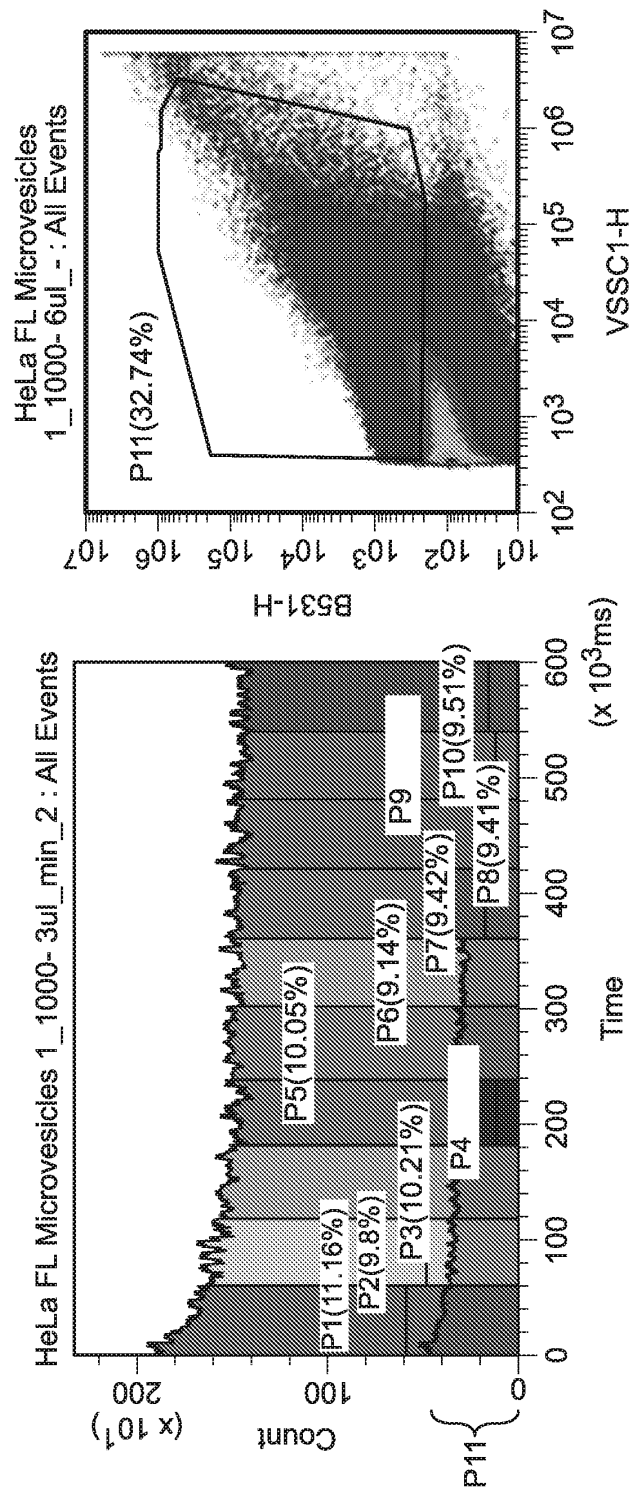
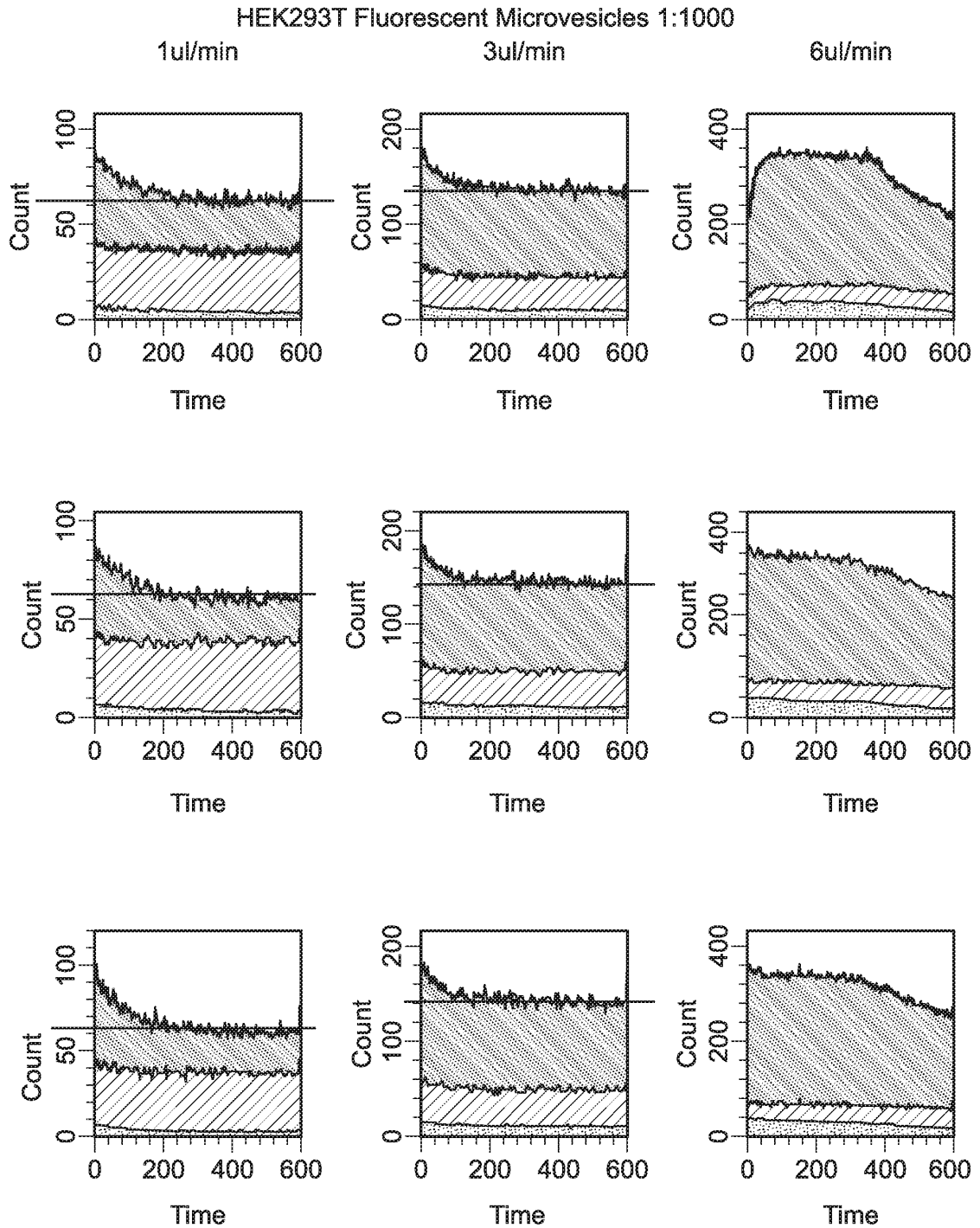


FIG. 9

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**FIG. 10**

SUBSTITUTE SHEET (RULE 26)

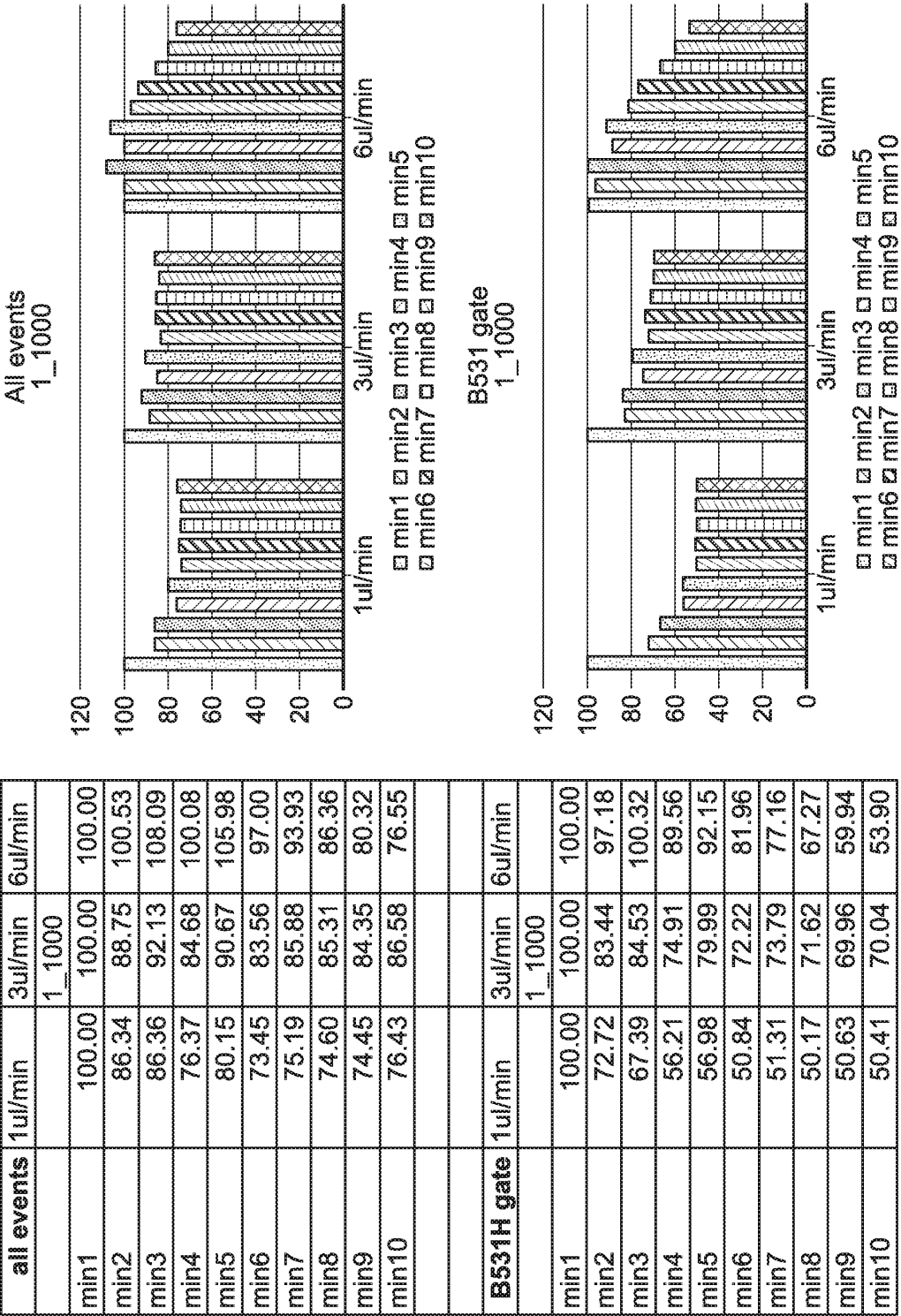
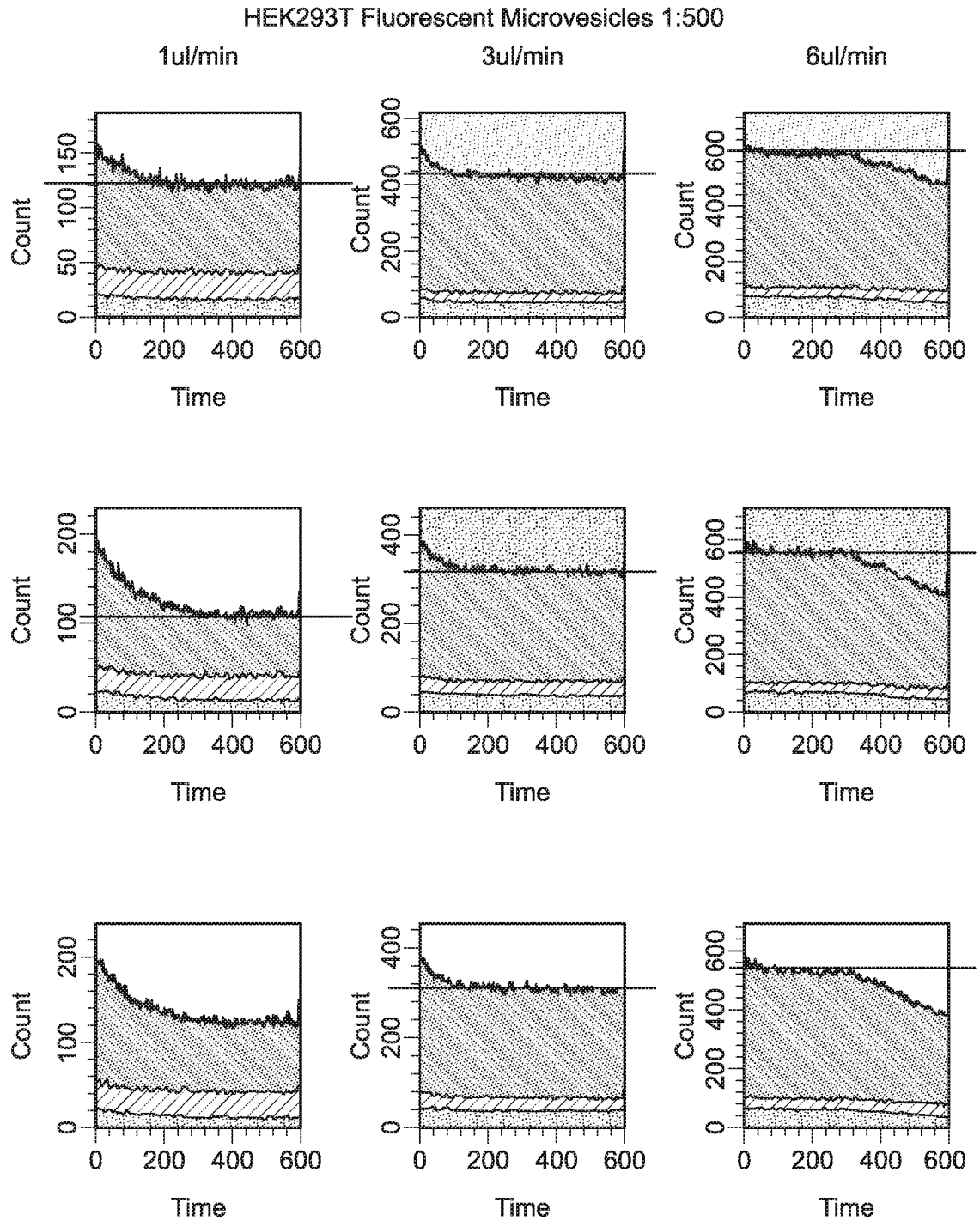


FIG. 11

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**FIG. 12**

SUBSTITUTE SHEET (RULE 26)

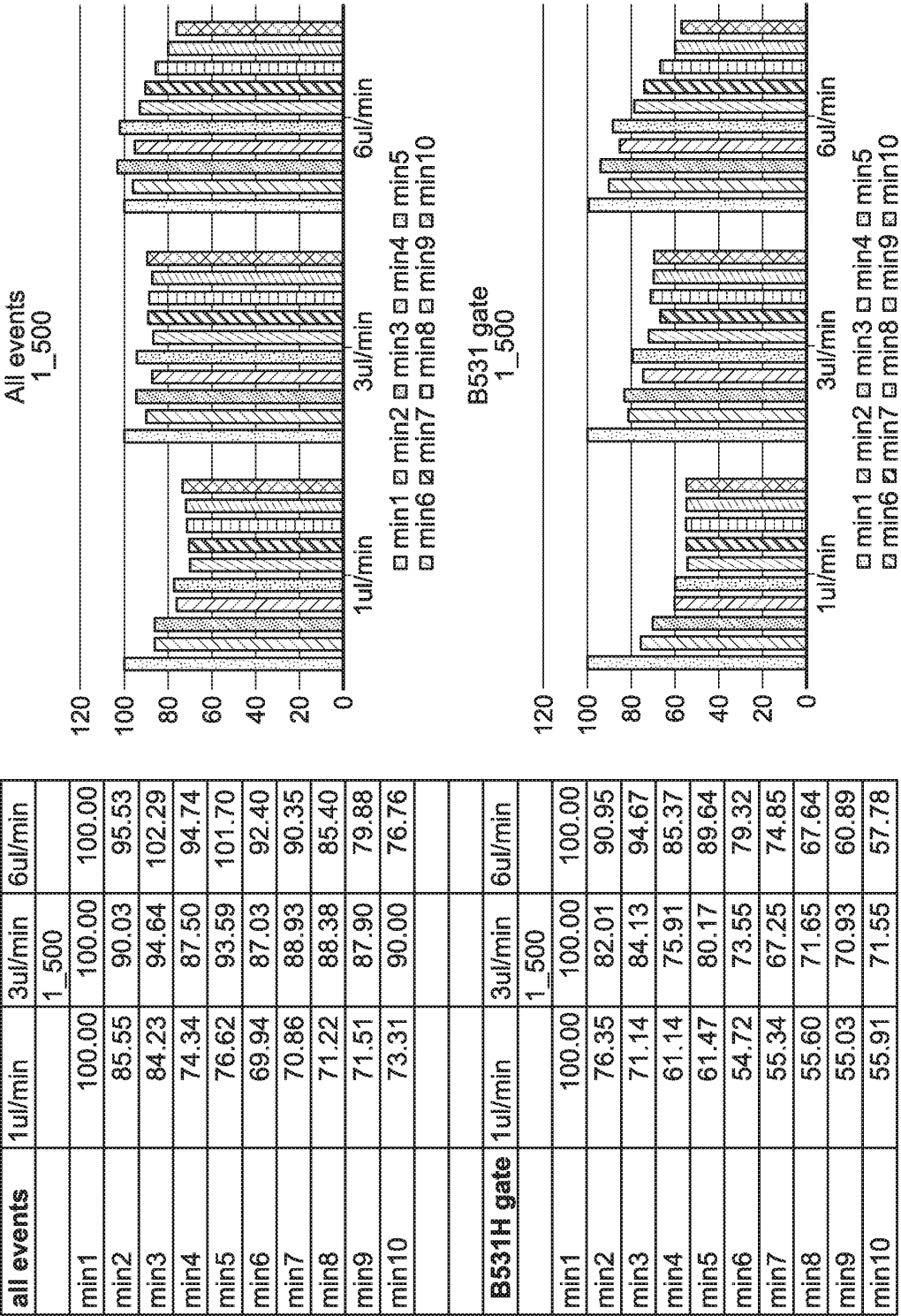
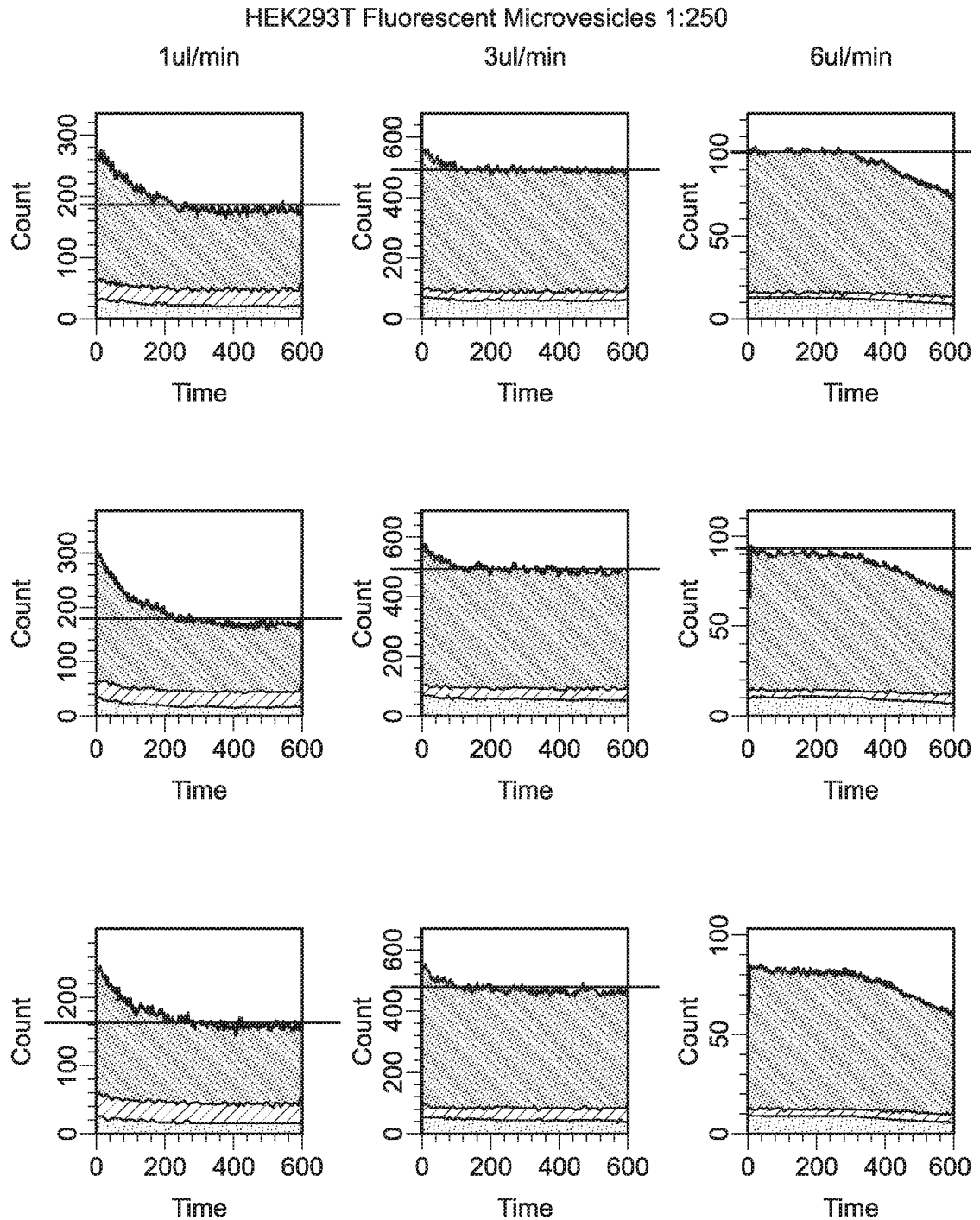


FIG. 13

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**FIG. 14**

SUBSTITUTE SHEET (RULE 26)

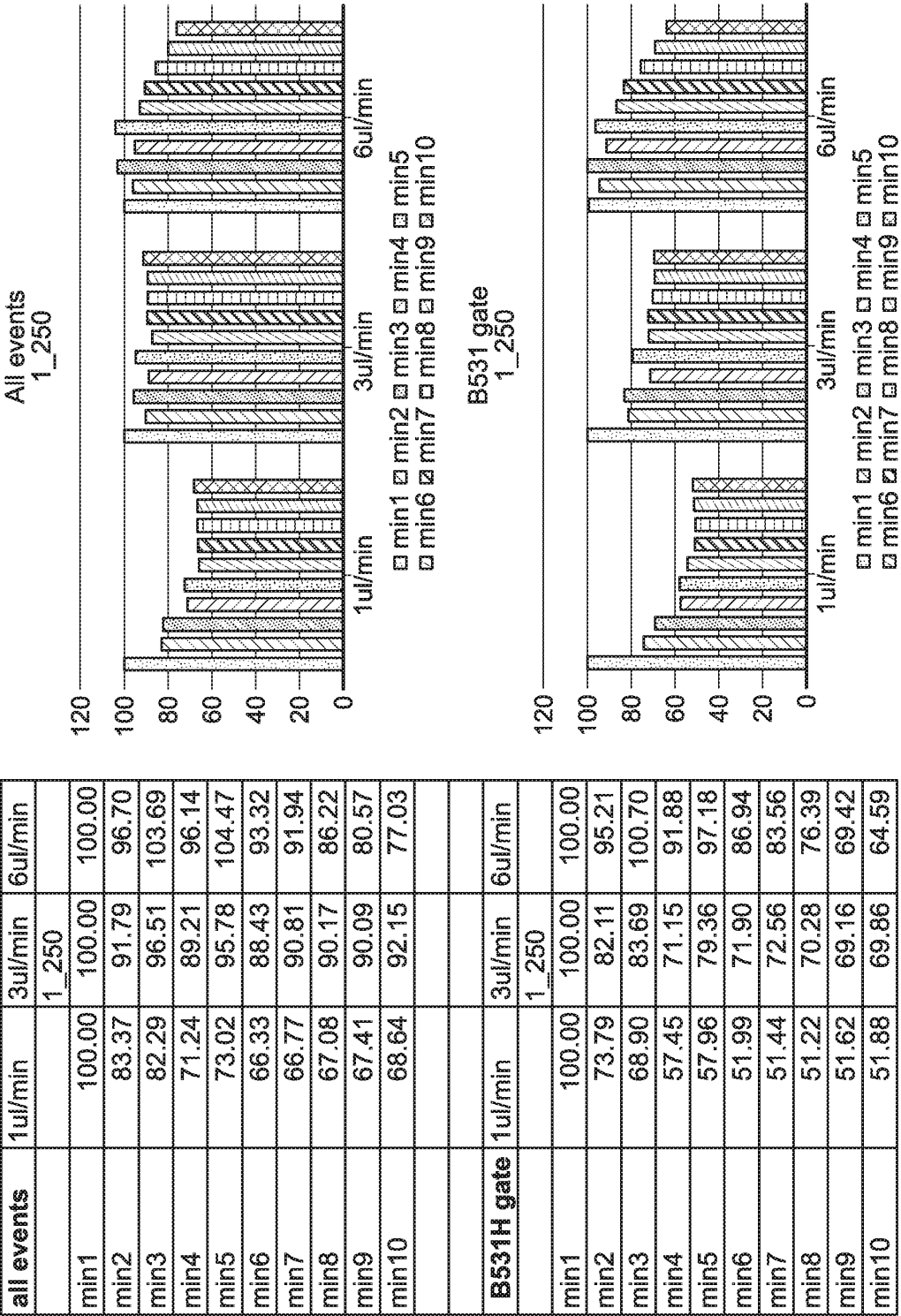


FIG. 15

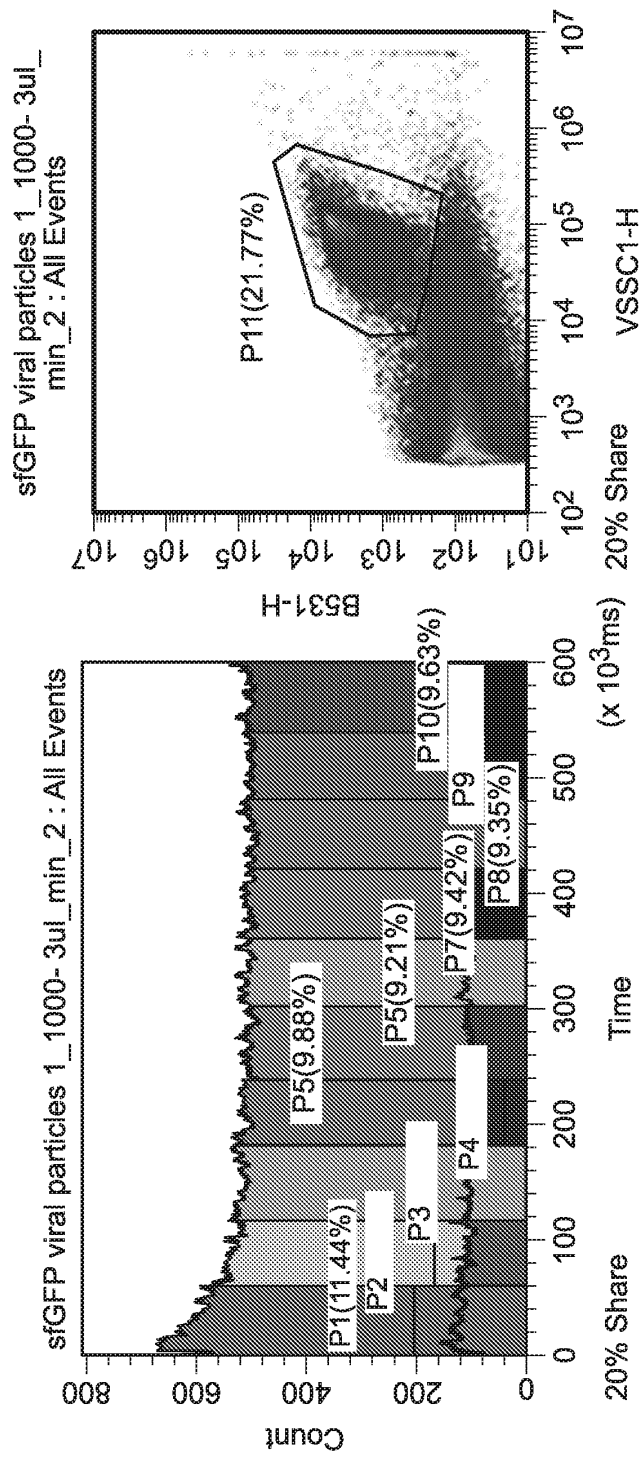


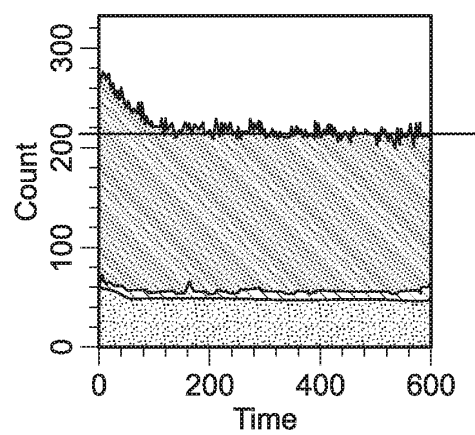
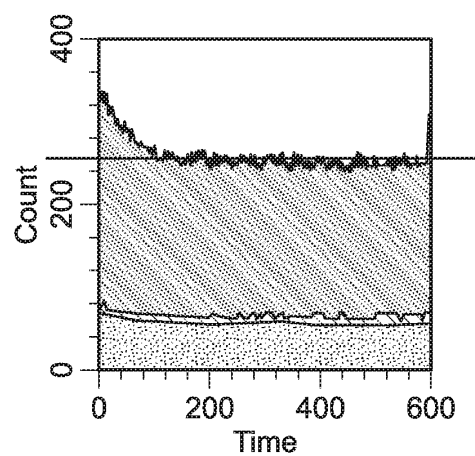
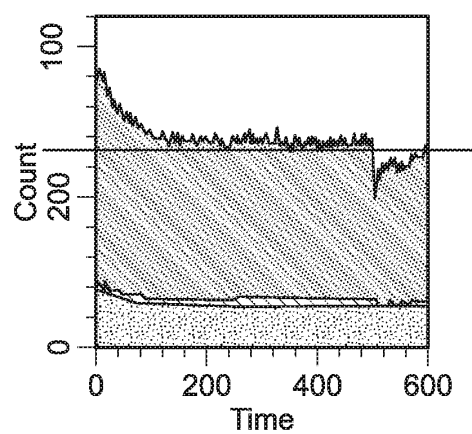
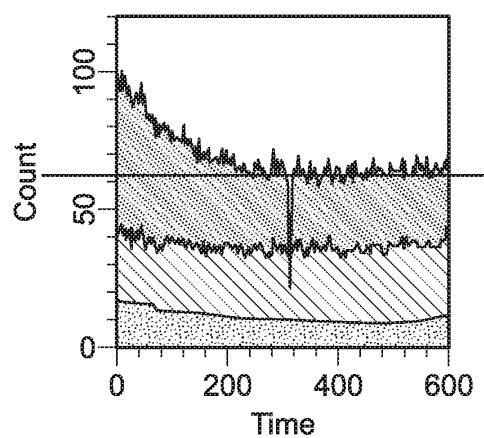
FIG. 16

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sfGFP 1_500

1ul/min

3ul/min

**FIG. 17**

SUBSTITUTE SHEET (RULE 26)

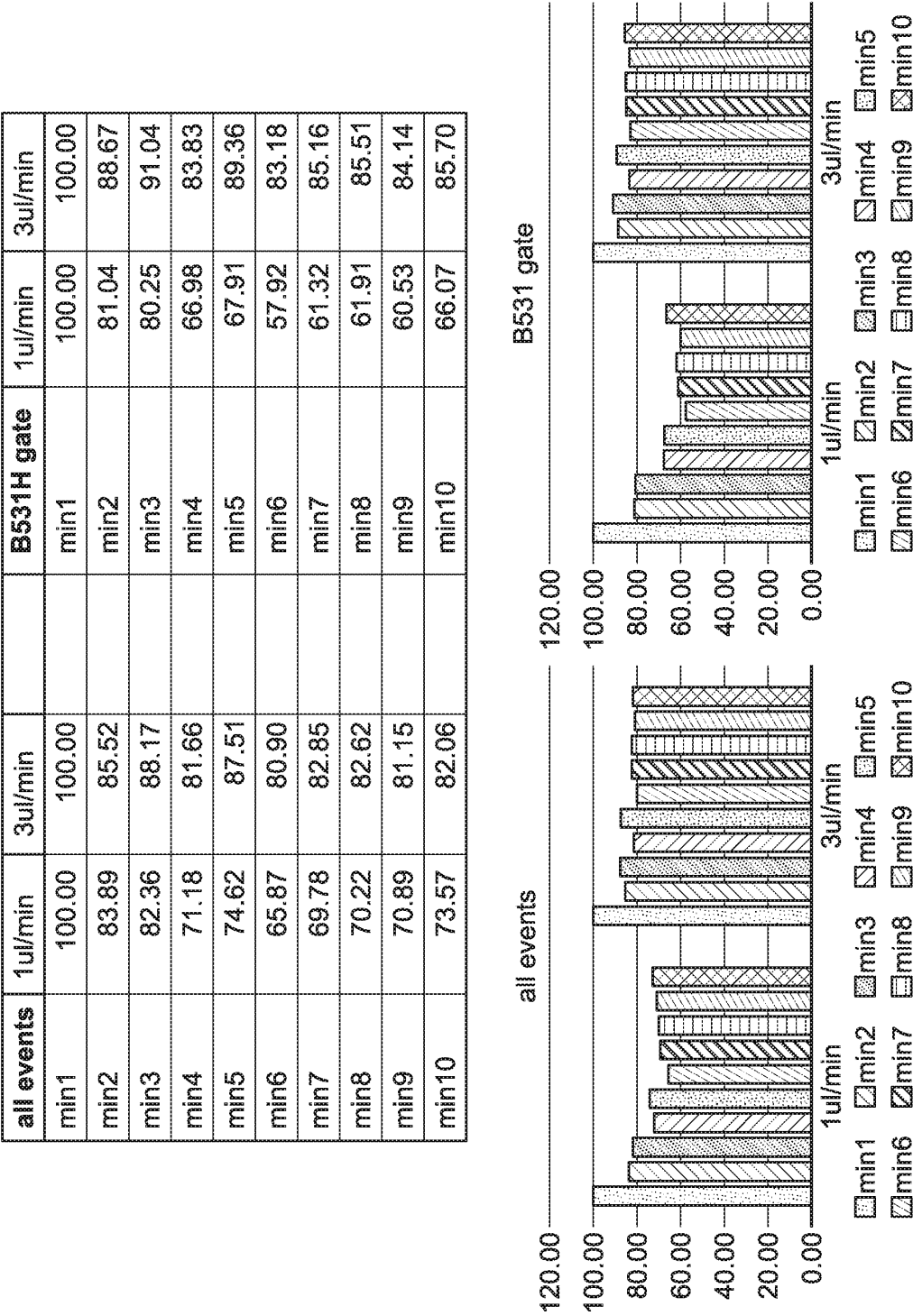


FIG. 18

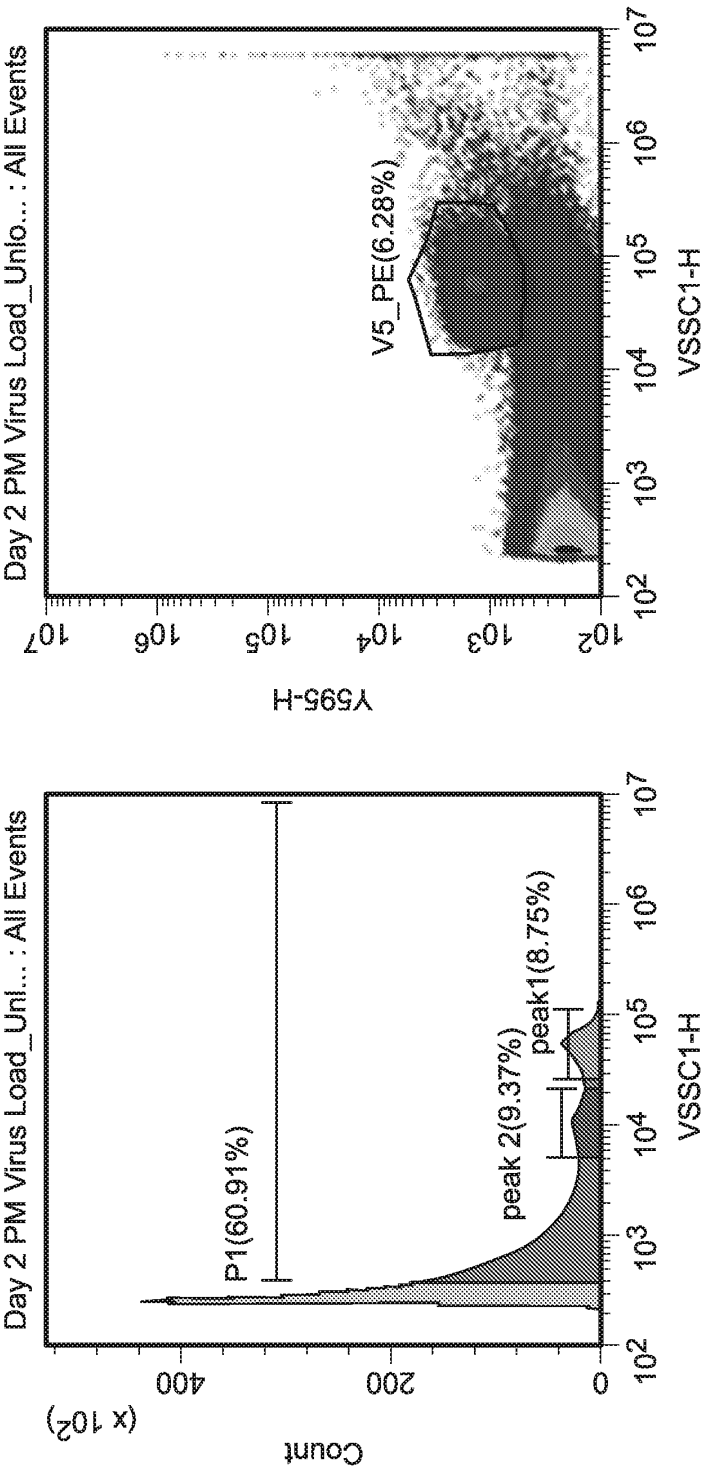


FIG. 19

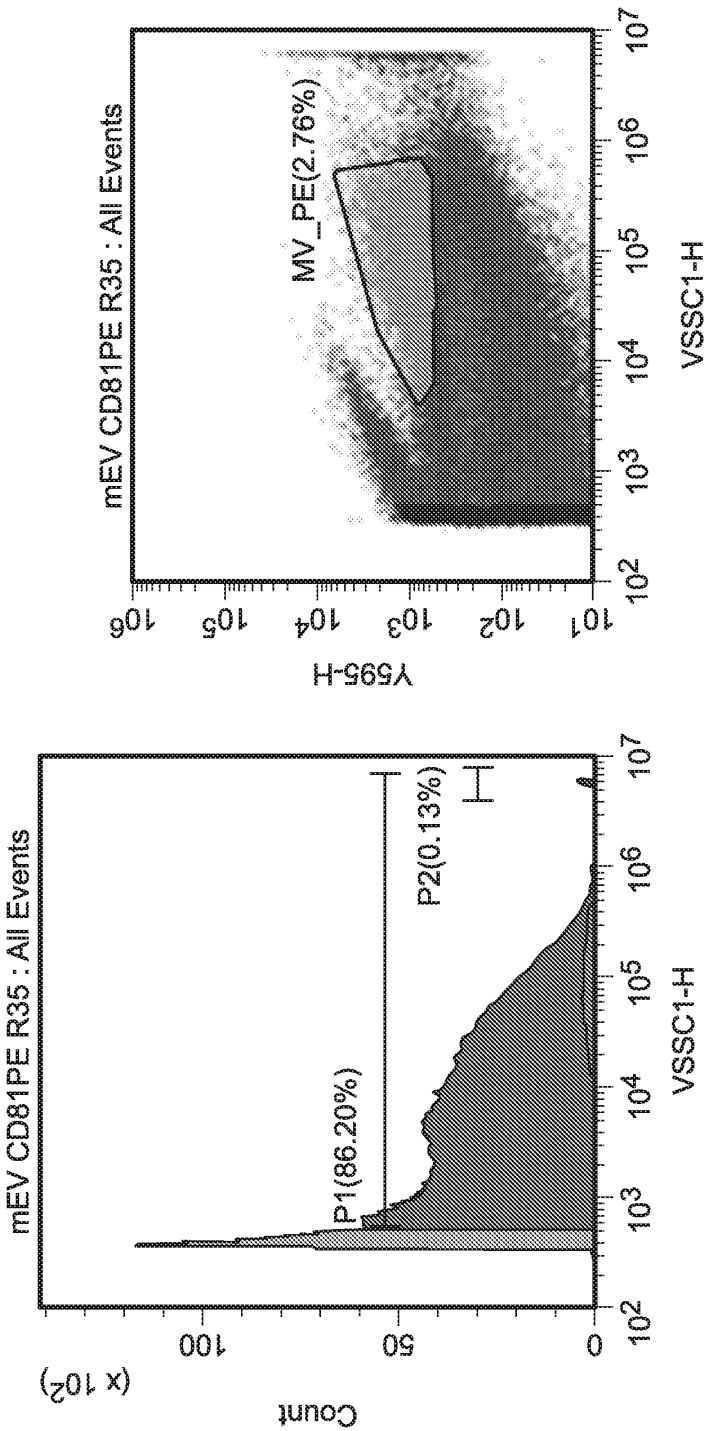


FIG. 20

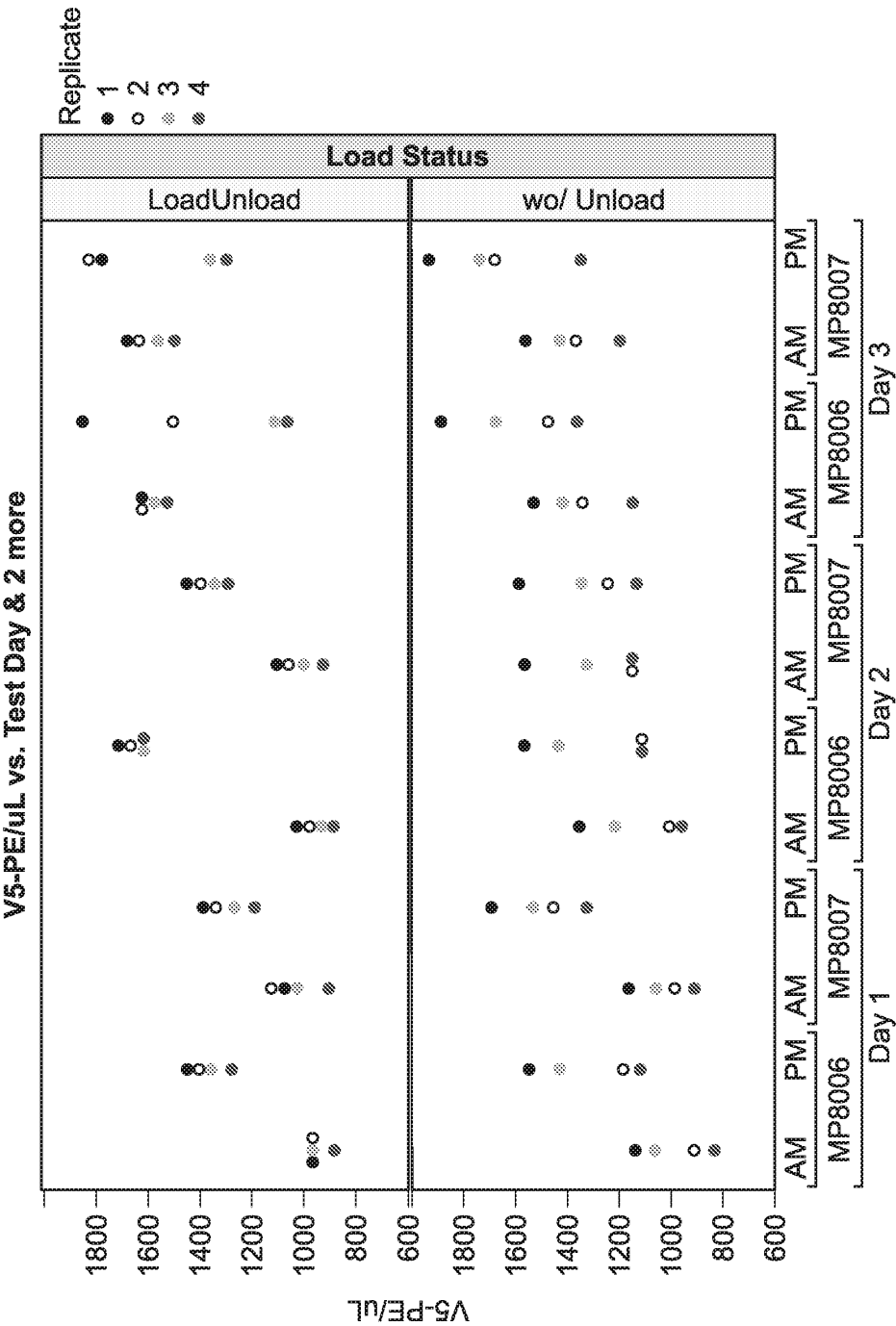
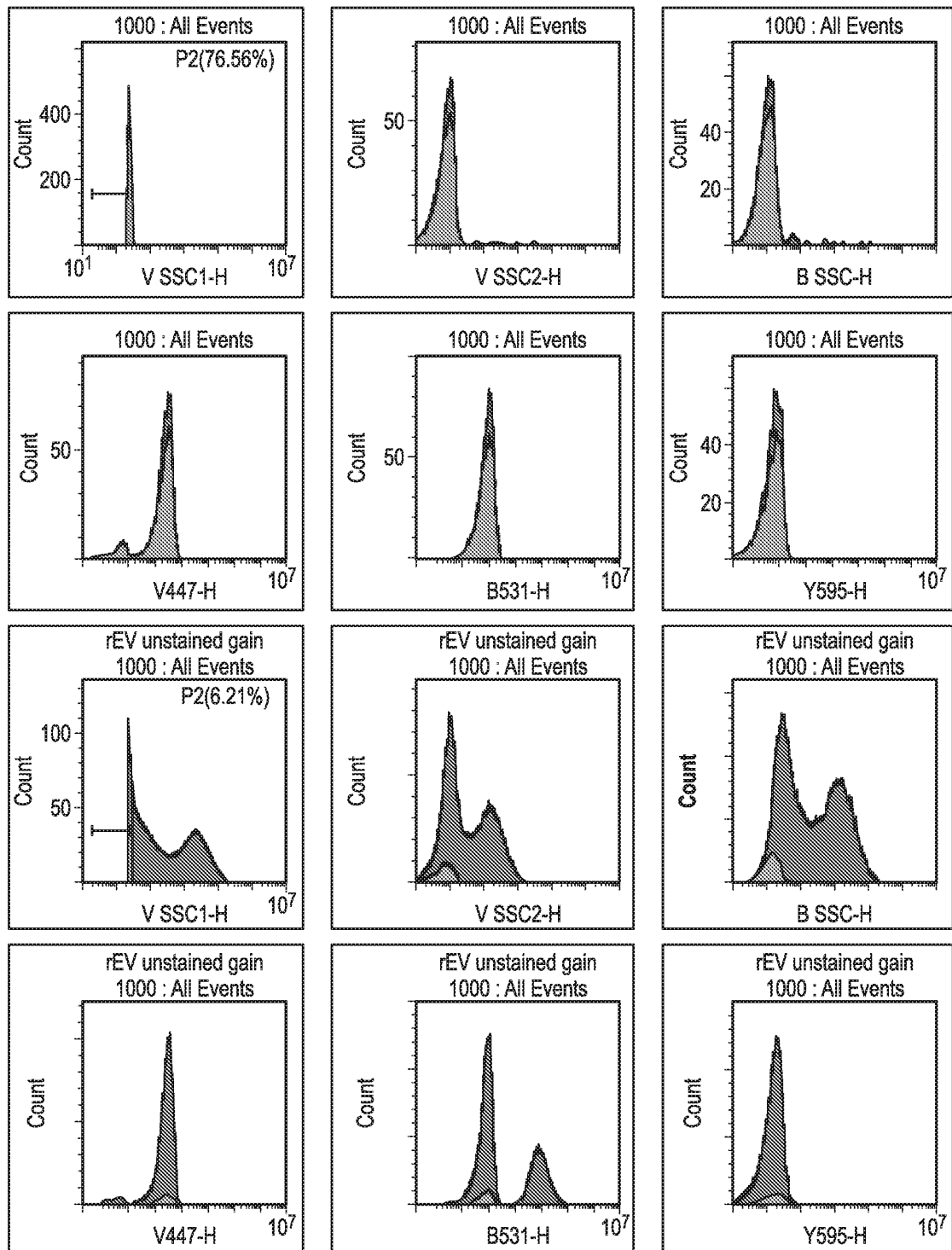
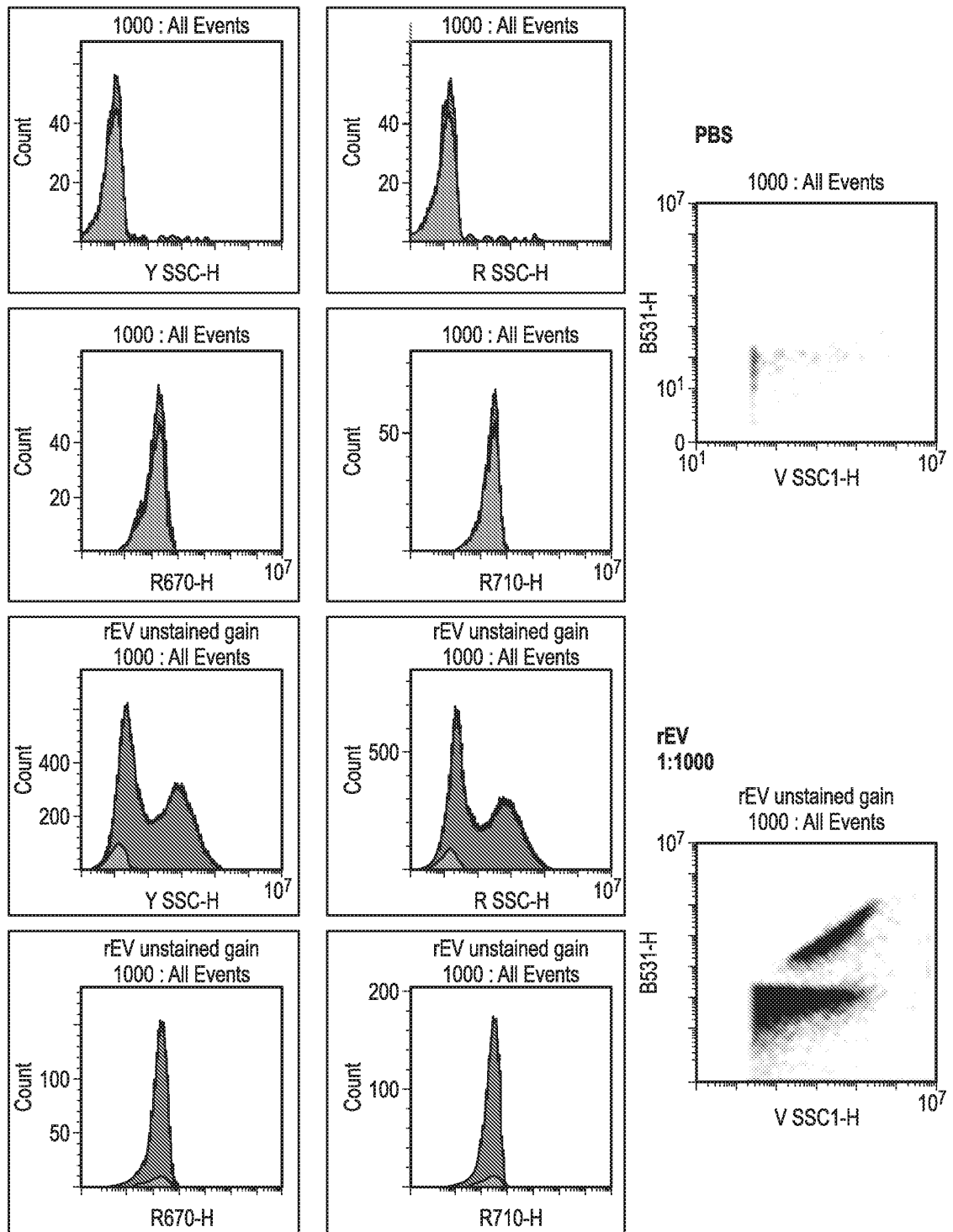


FIG. 21

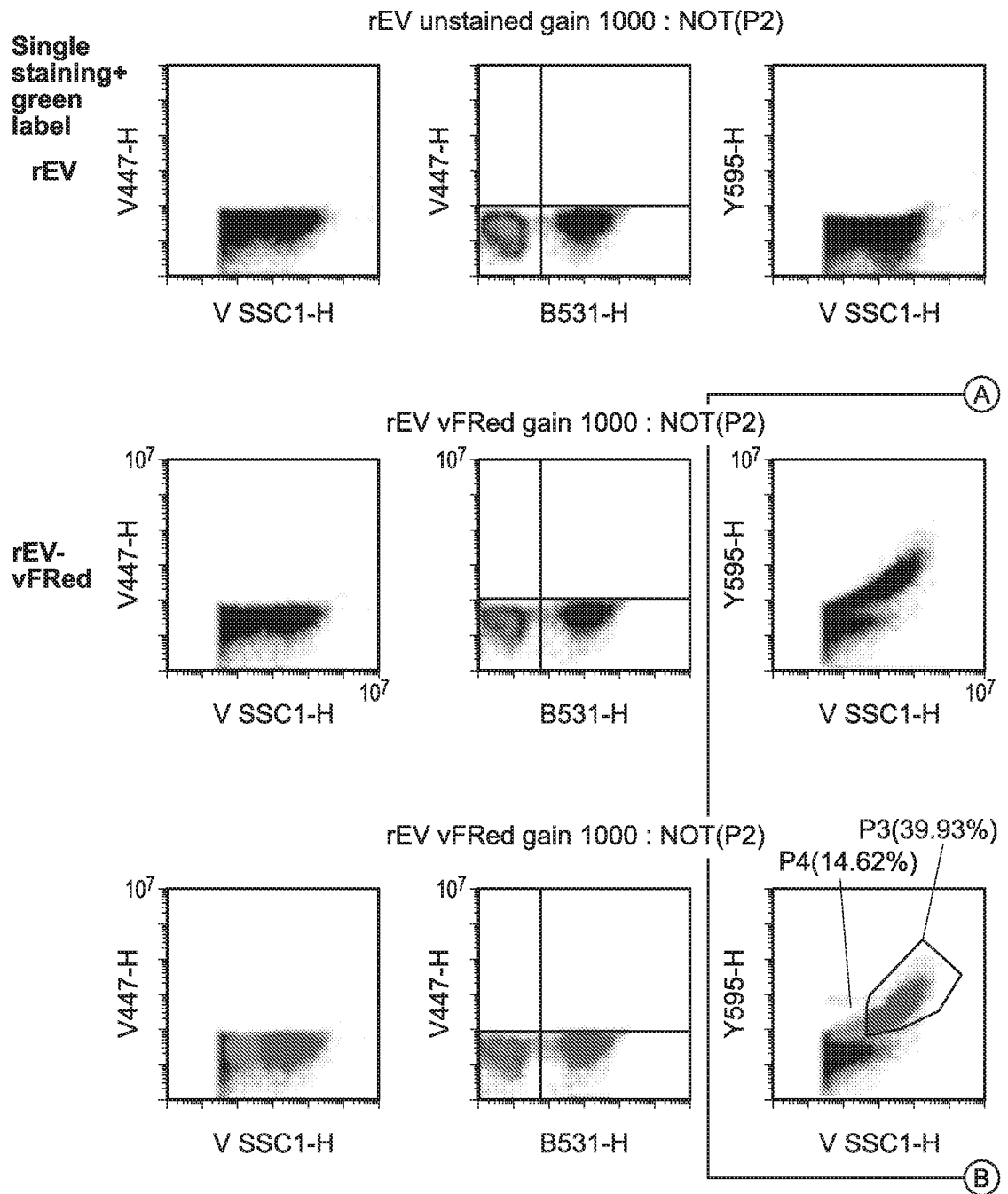
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**FIG. 22**

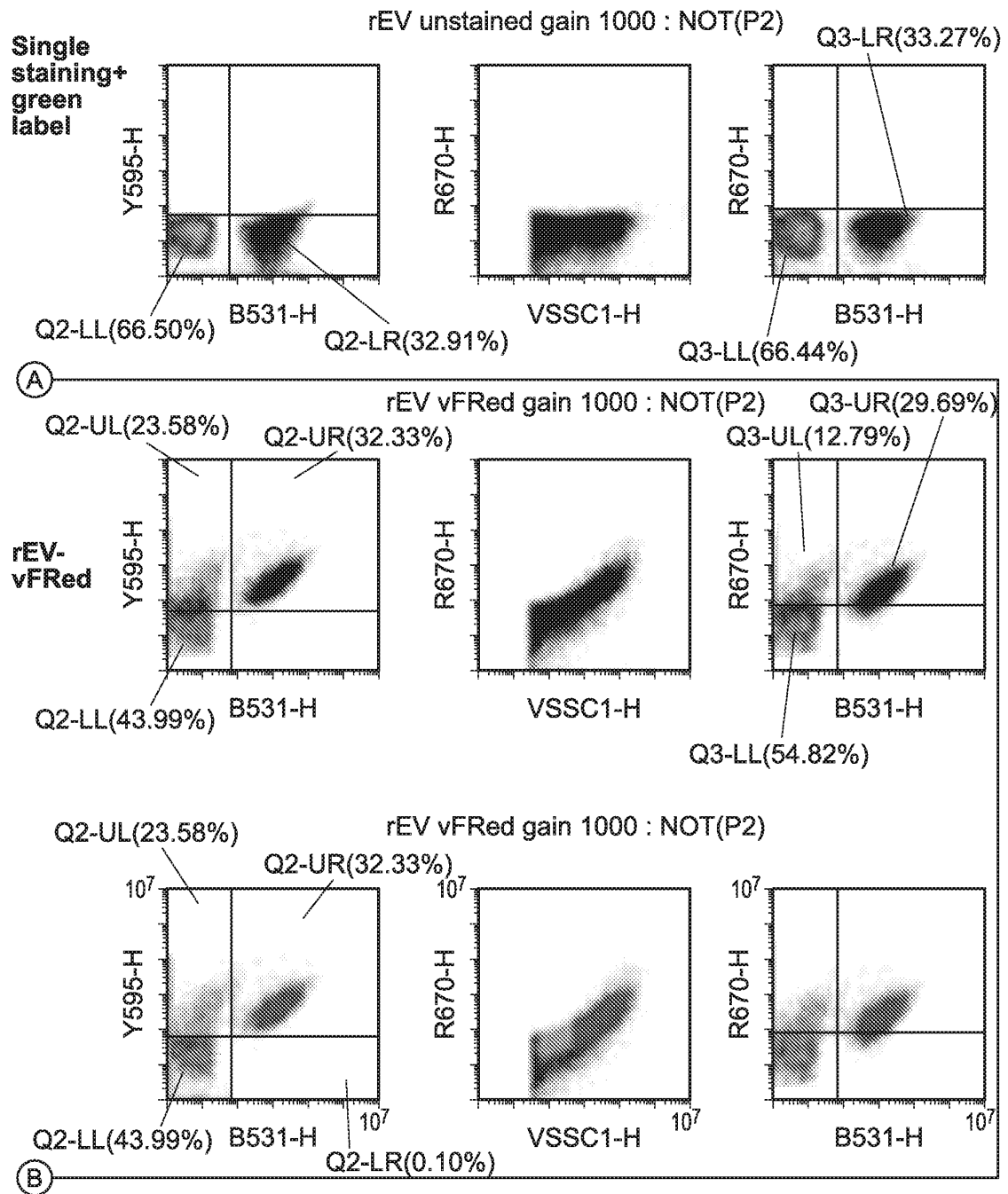
24/127

**FIG. 22 (Cont.)**

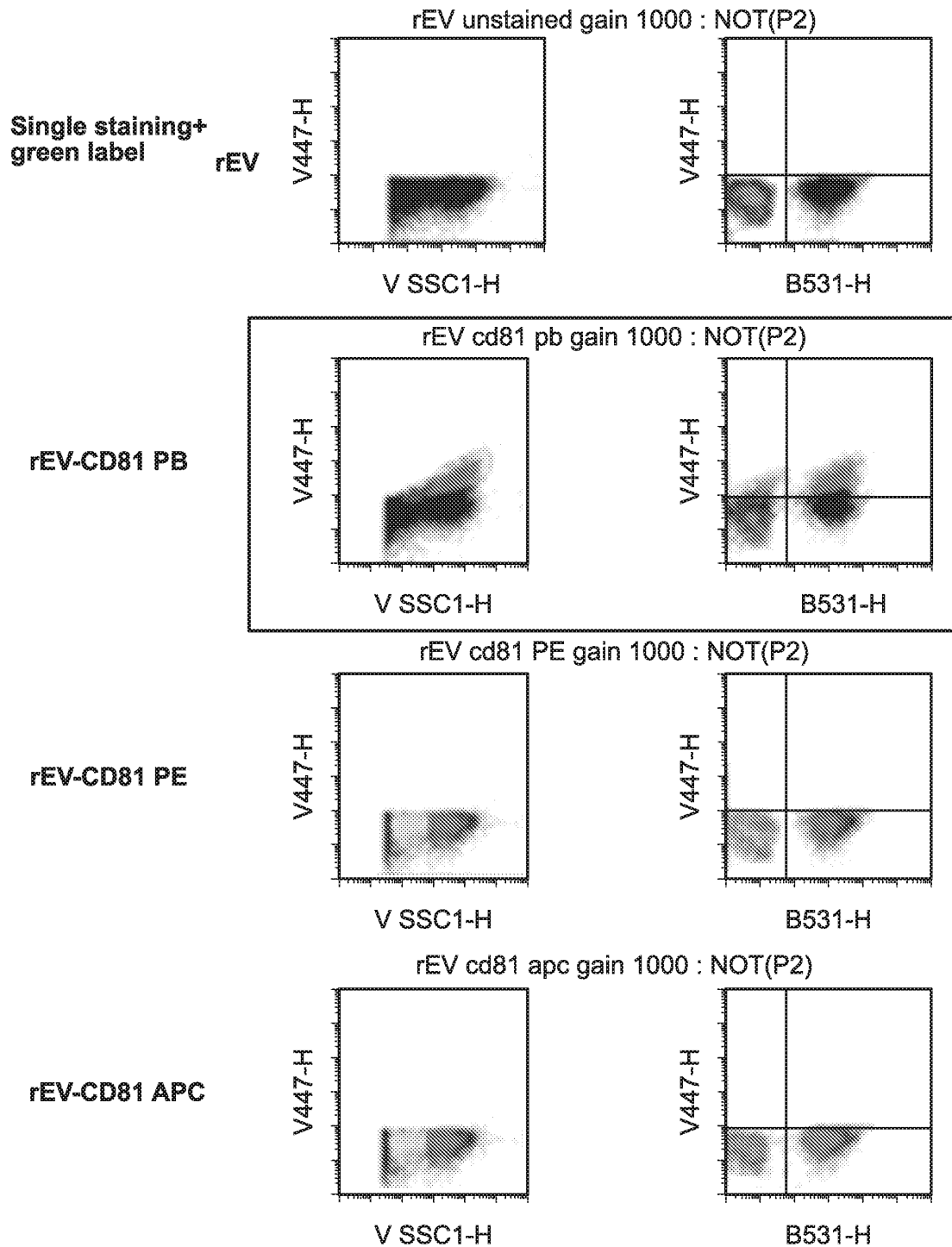
25/127

**FIG. 23**

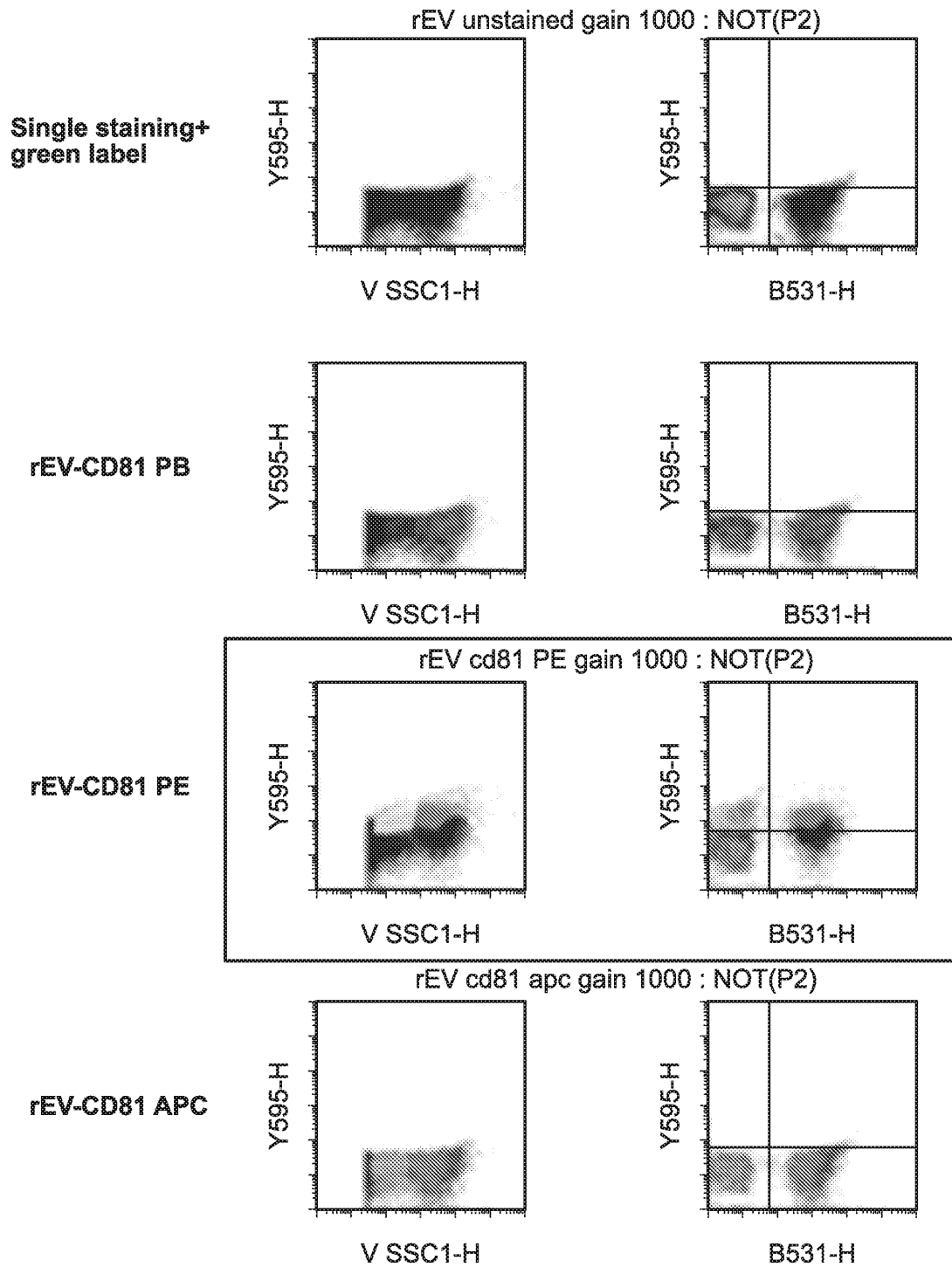
26/127

**FIG. 23 (Cont.)**

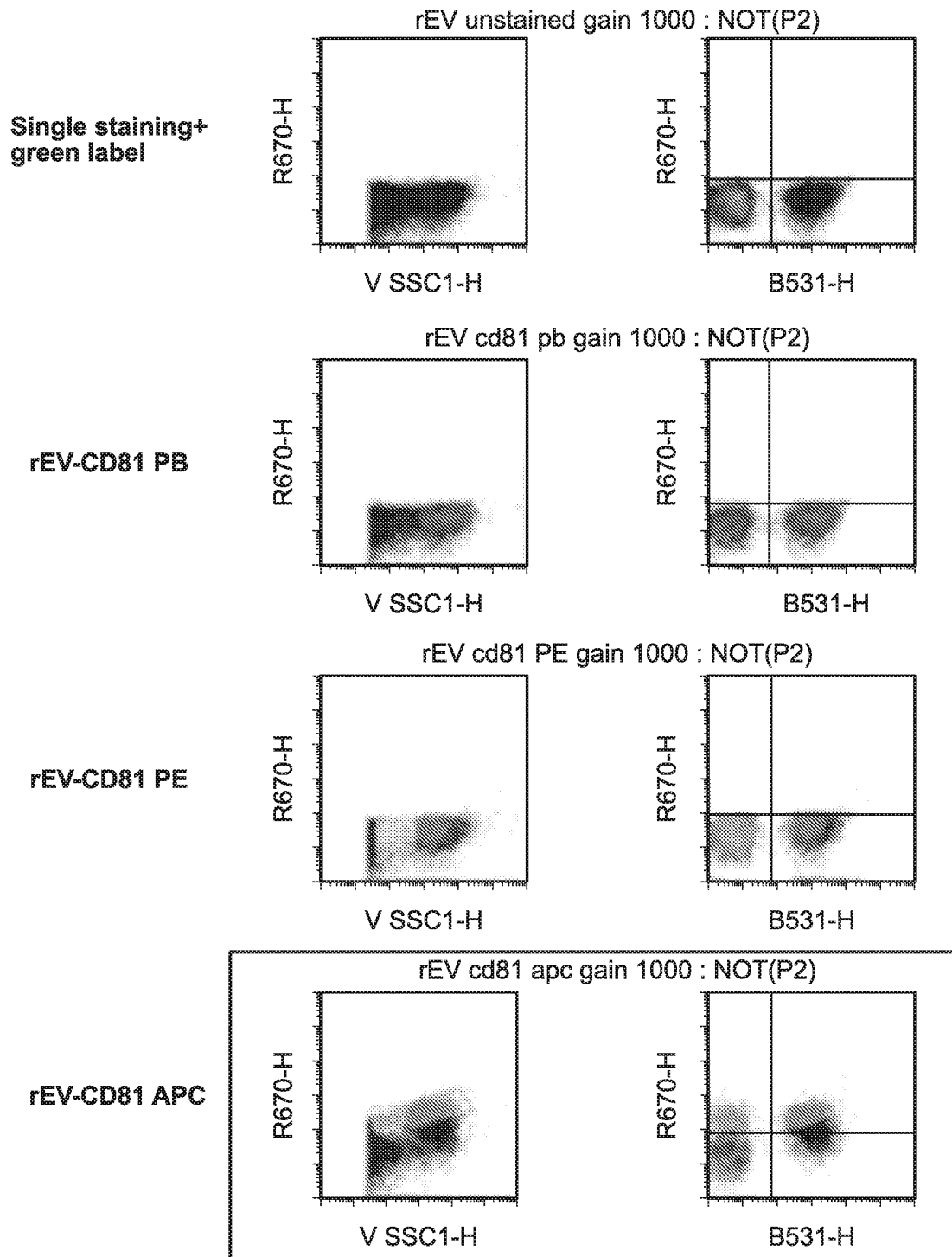
27/127

**FIG. 24**

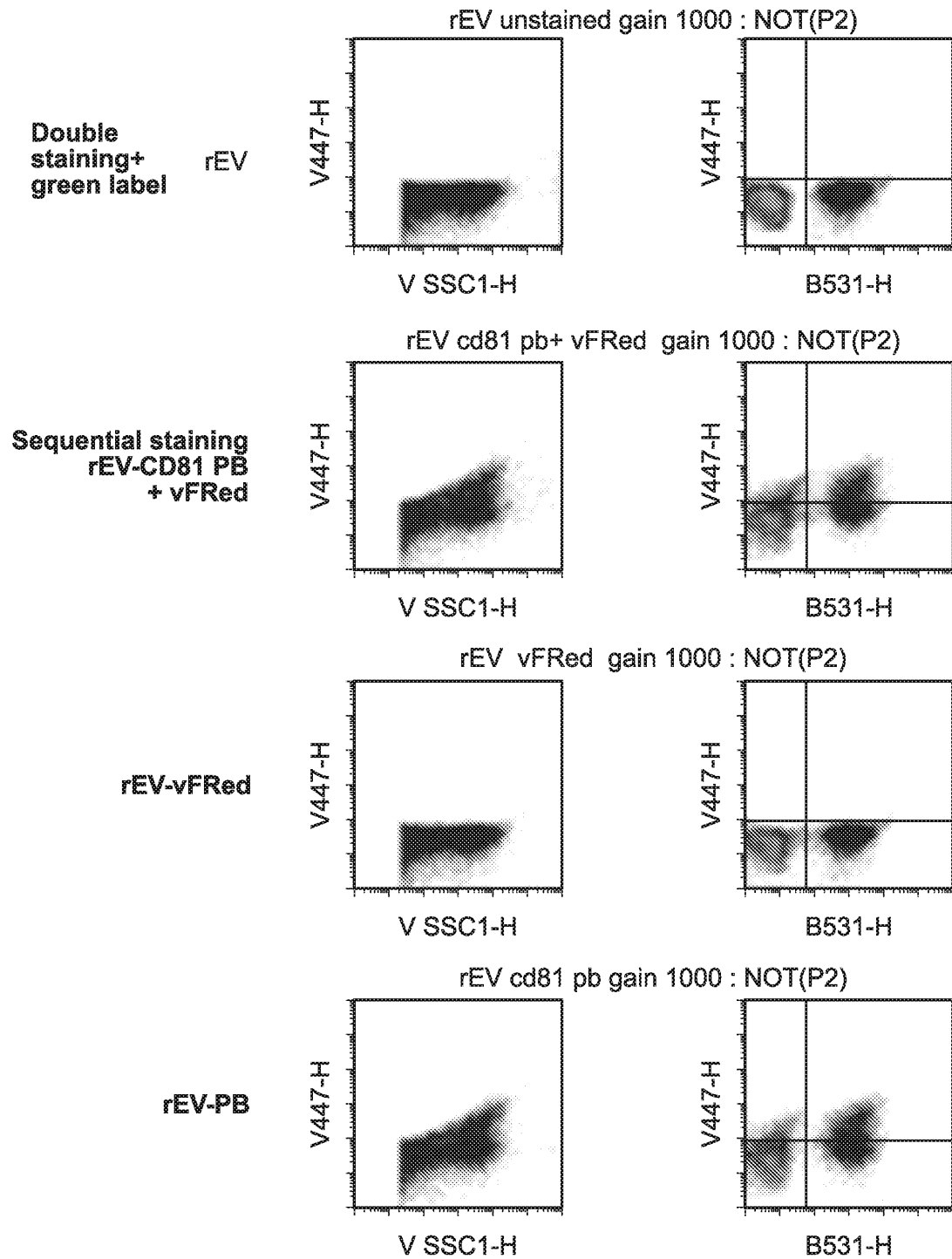
28/127

**FIG. 24 (Cont. 1)**

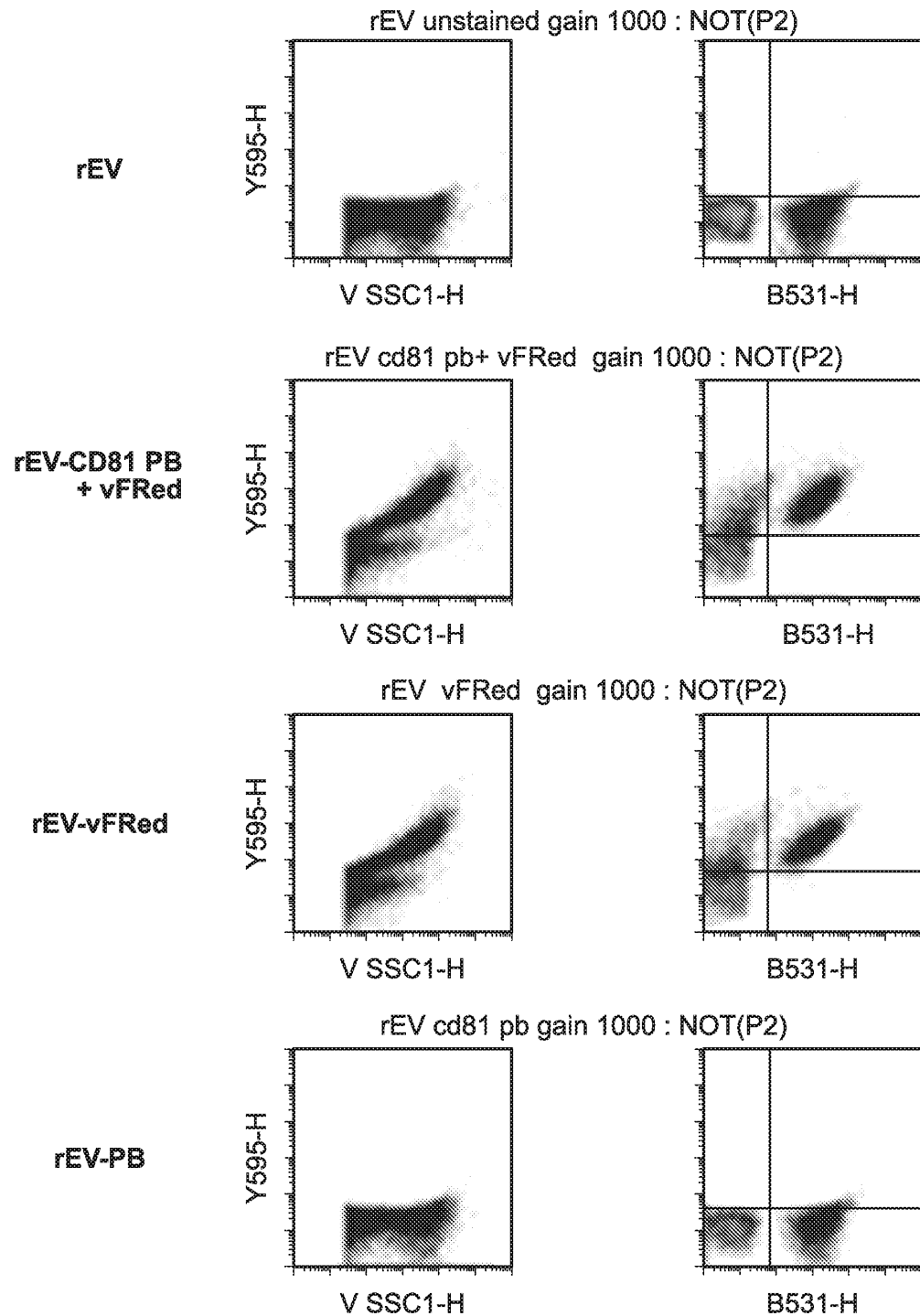
29/127

**FIG. 24 (Cont. 2)**

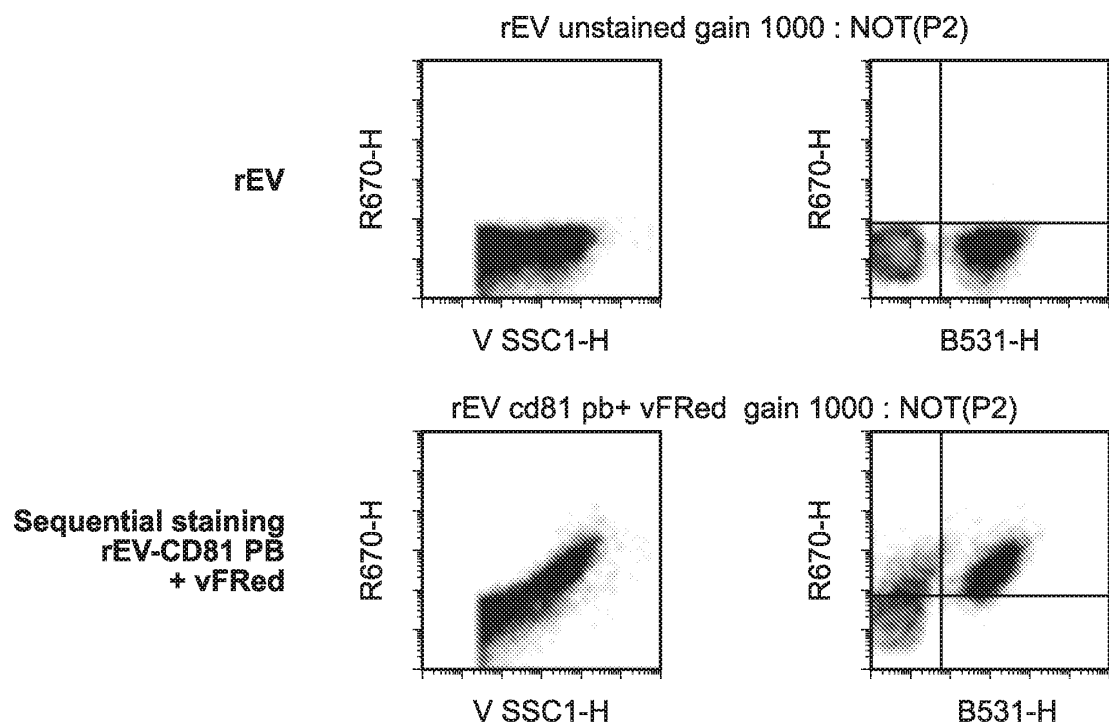
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**FIG. 25**

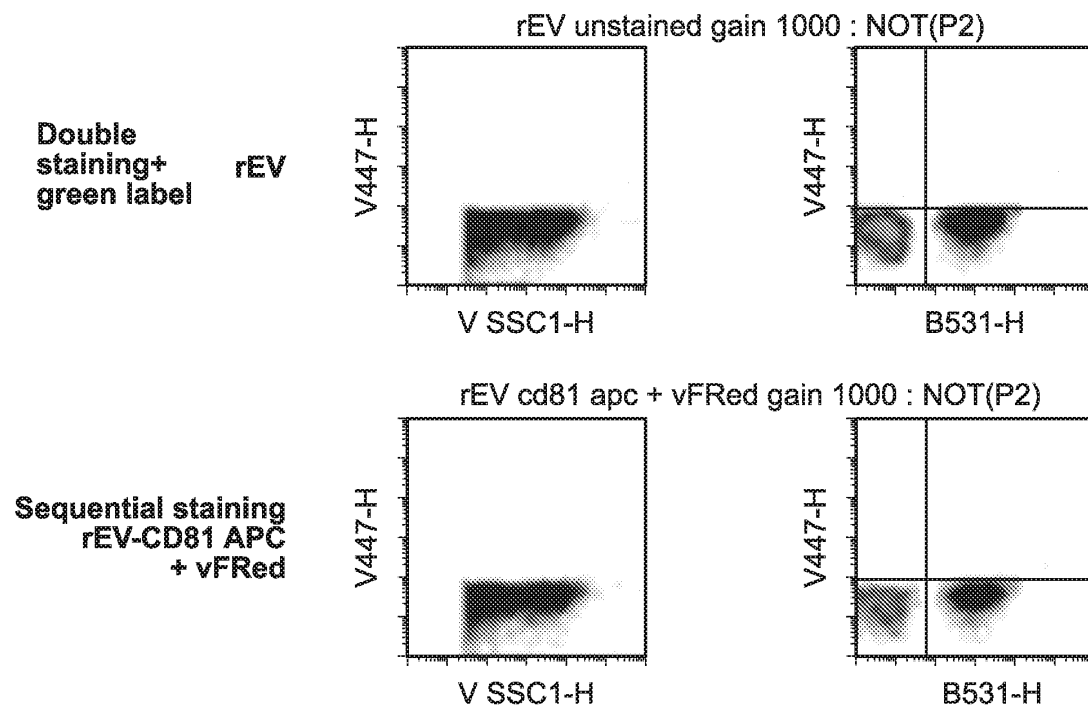
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**FIG. 25 (Cont. 1)**

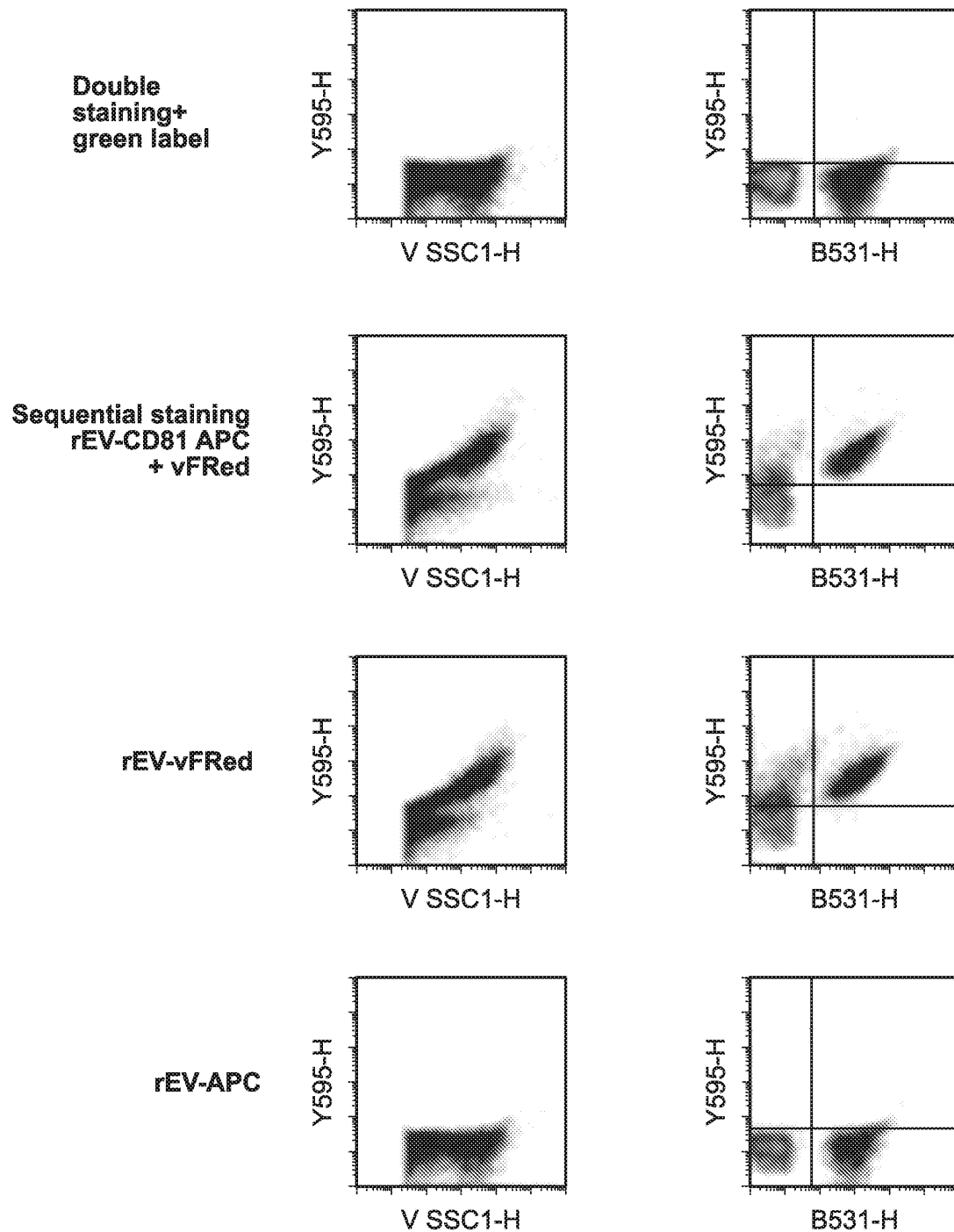
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**FIG. 25 (Cont. 2)**

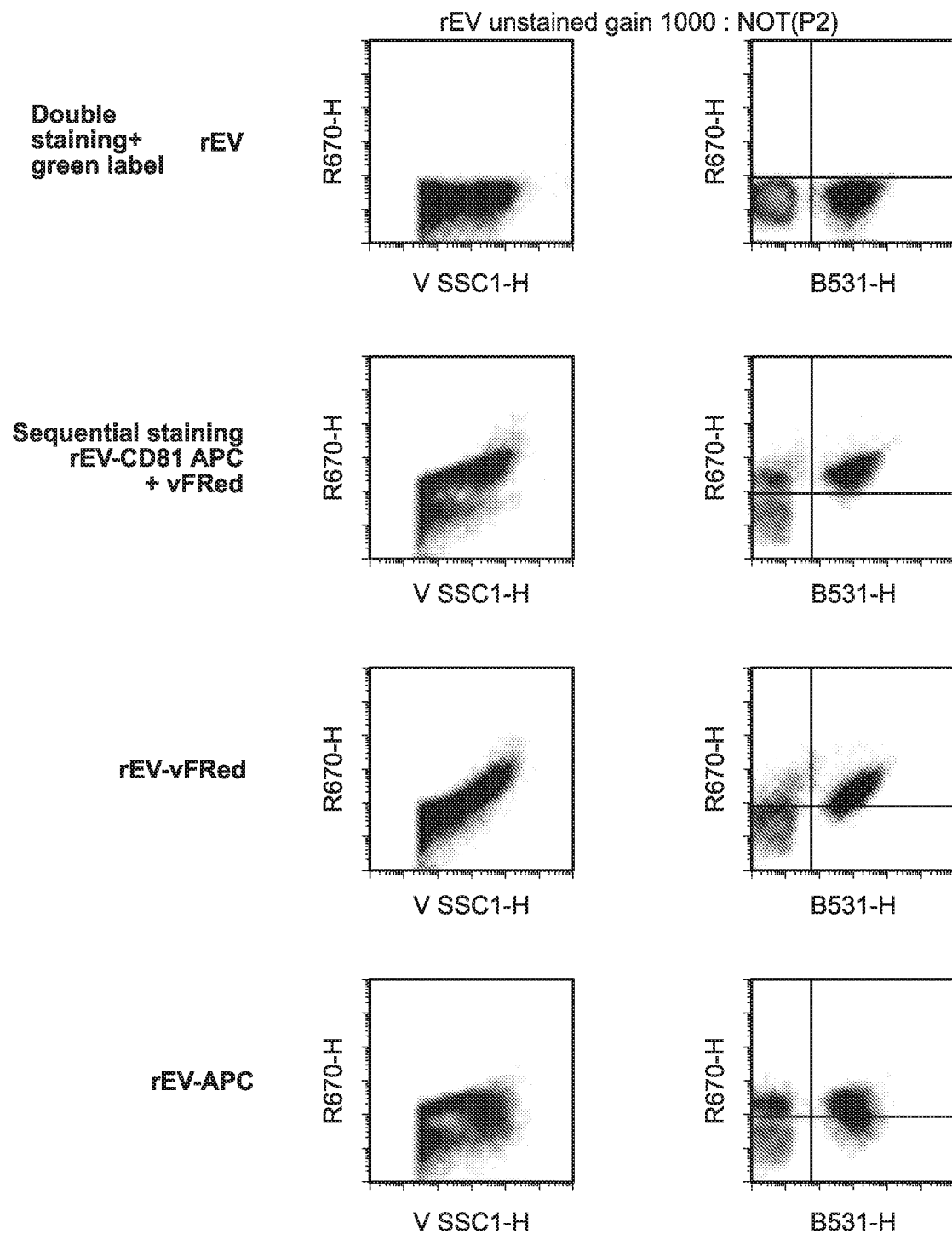
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**FIG. 26**

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**FIG. 26 (Cont. 1)**

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**FIG. 26 (Cont. 2)**

Antibodies background

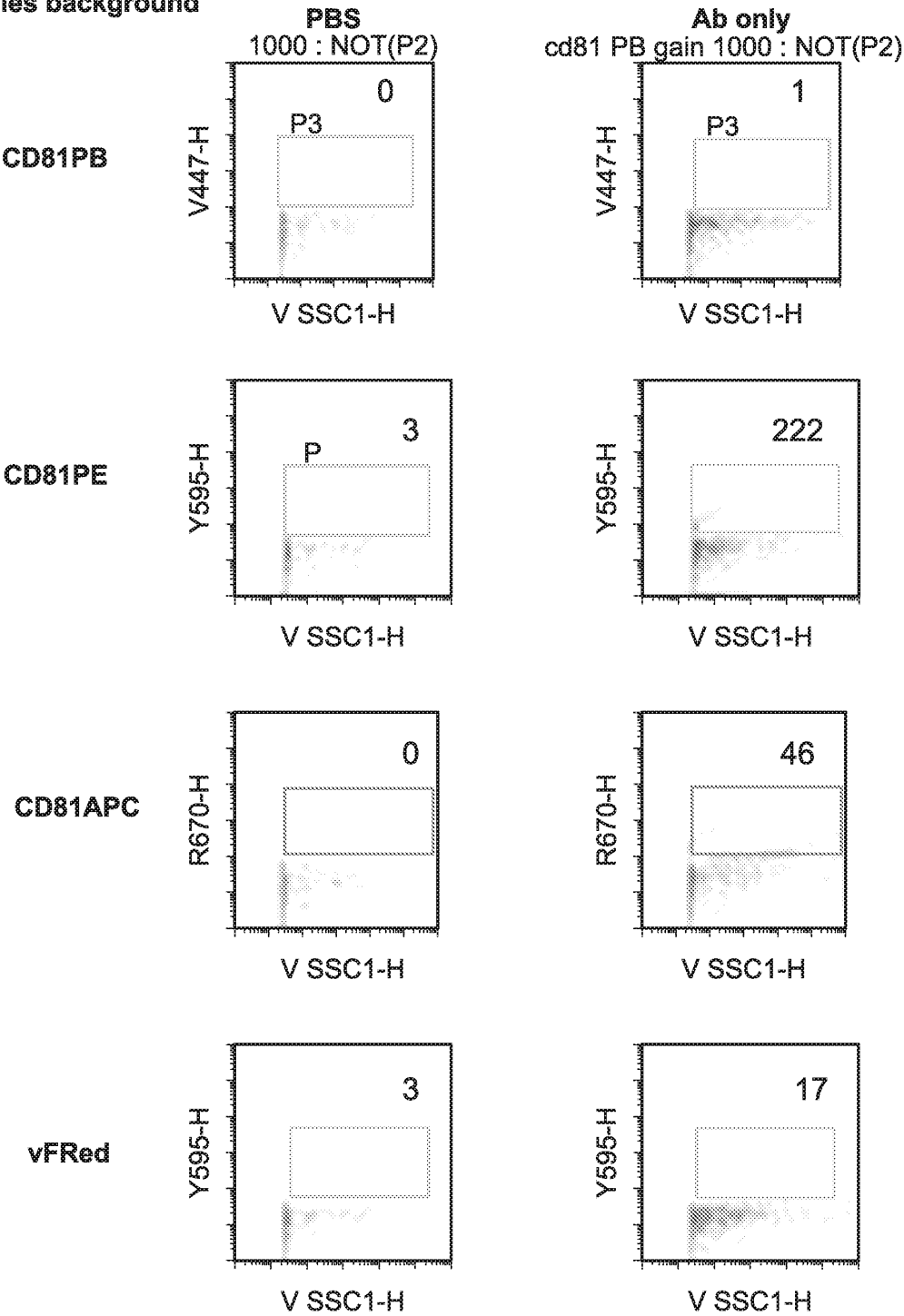


FIG. 27

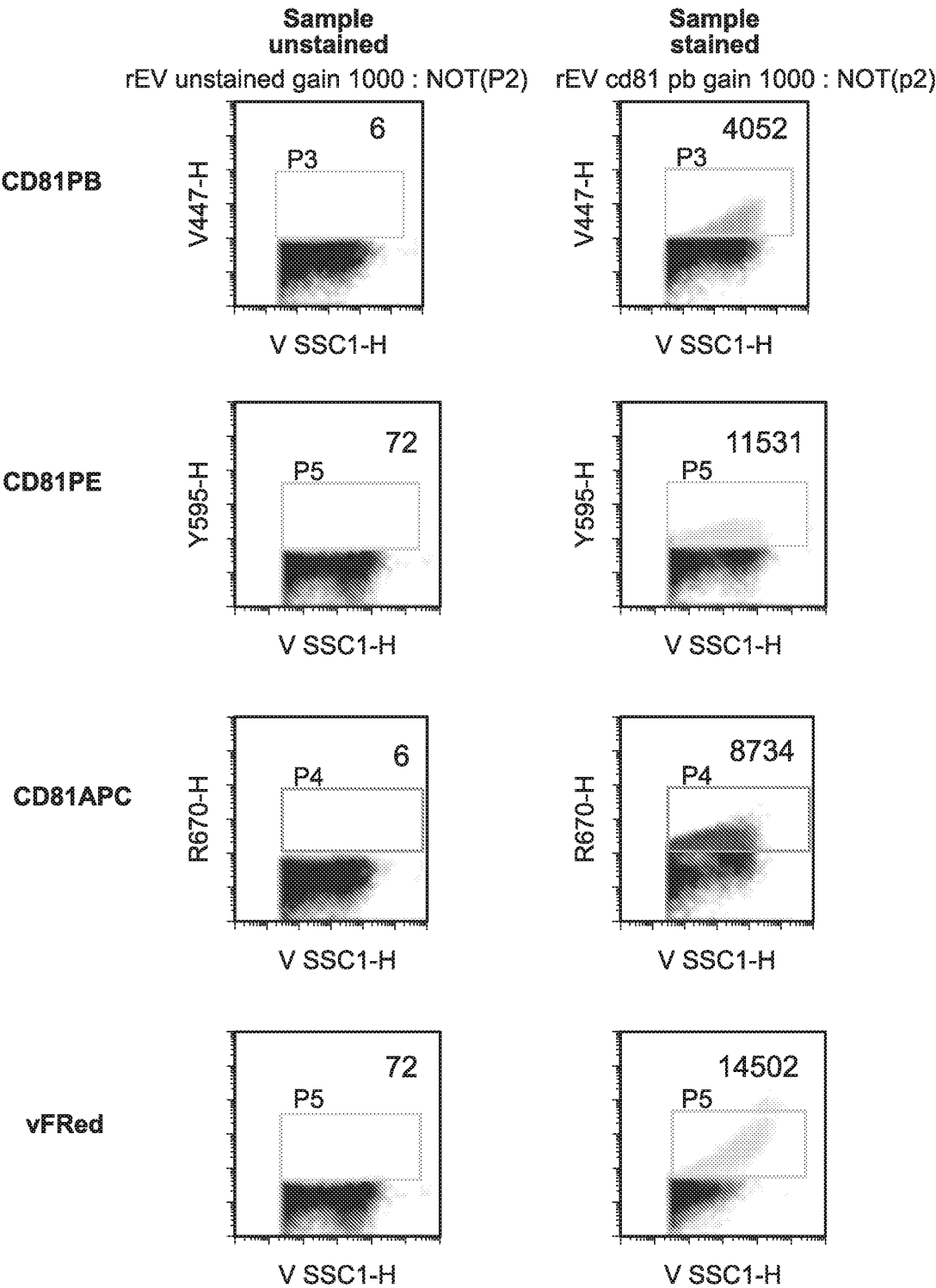


FIG. 27 (Cont.)

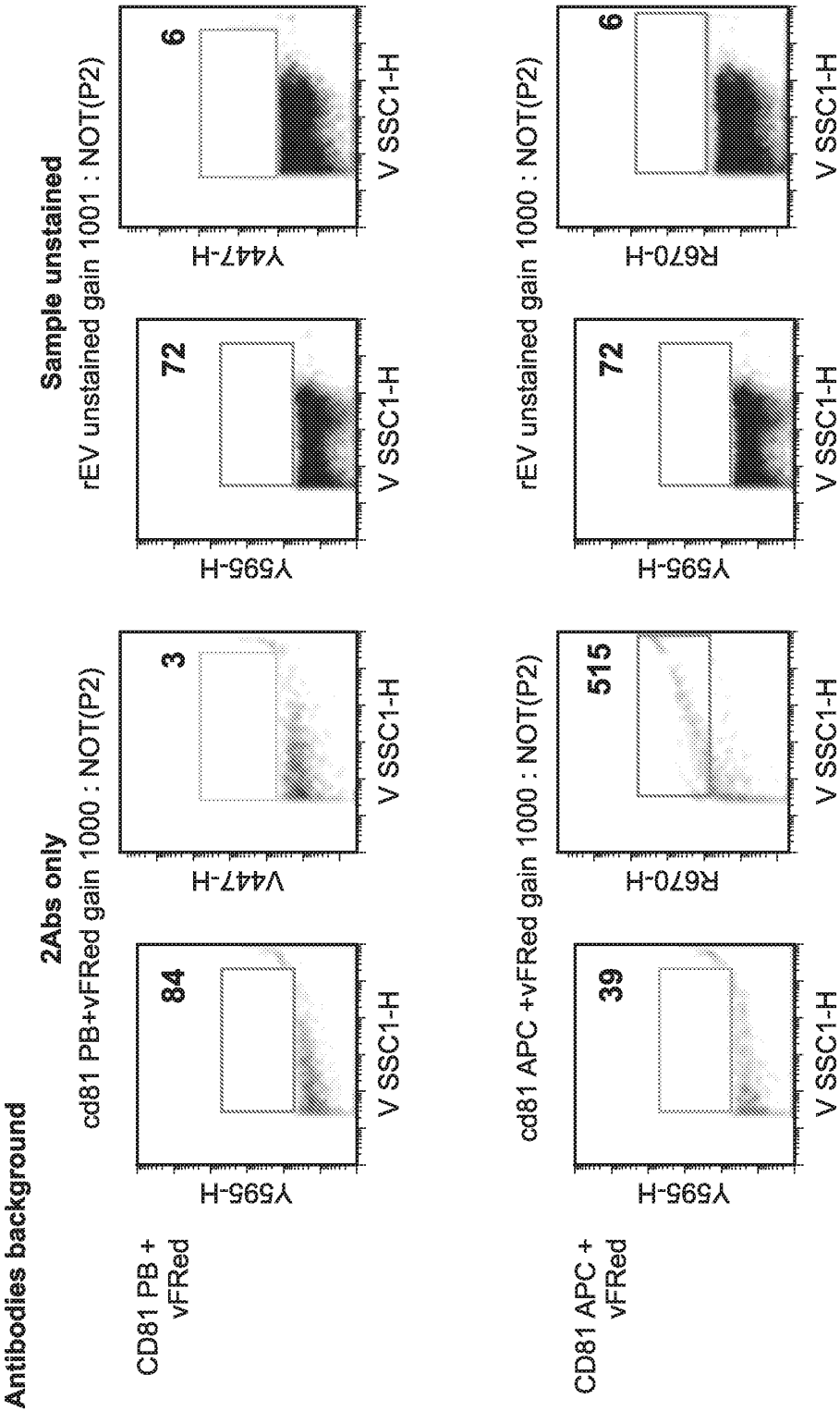


FIG. 28

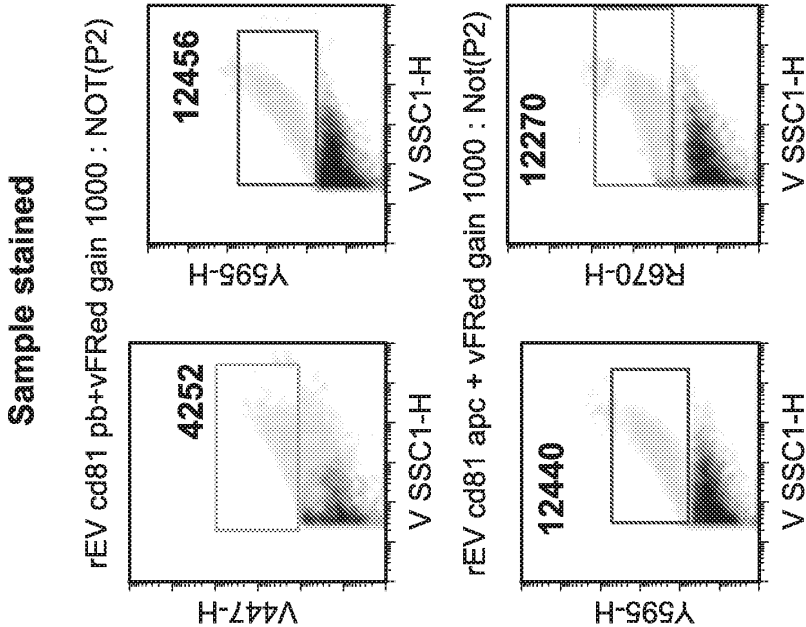


FIG. 28 (Cont.)

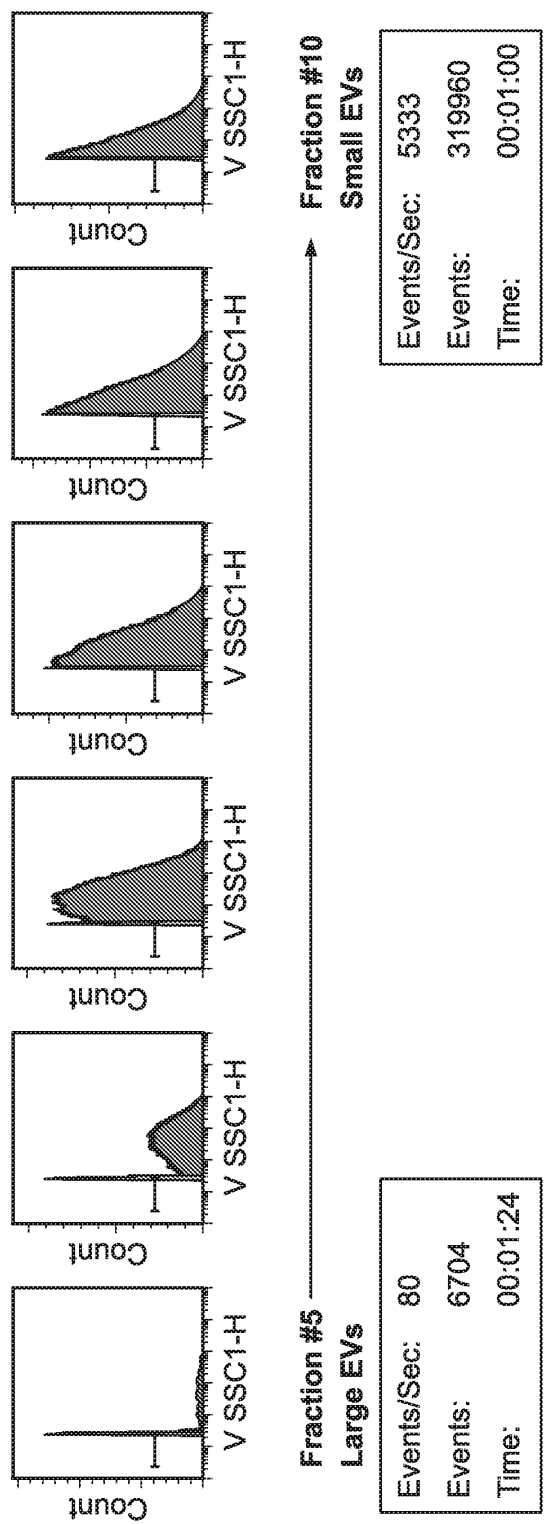


FIG. 29

Acq. Setting - PPP F12 MIX 1_500

Gain

Threshold

Width

Primary Threshold (Trigger Level)

Channel: V SSC1

Manual: 250 (>0) Height

Compensation Matrix - PPP F12 MIX 1_500

☒ Use ☒ Show Autofluorescence ☐ Area and Height in Sync

Height

Autofl	Channel	-V447%	-B531%	-Y595%	-R670%	-R710%	-R792%
0.00	V447		0.00	0.00	0.00	0.00	0.00
0.00	B531	25.00		0.00	0.00	0.00	1.00
0.00	Y595	0.66	2.71		0.00	0.00	15.00
0.00	R670	0.00	0.00	0.00		0.00	0.00
0.00	R710	0.00	0.00	0.00	25.06		0.00
0.00	R792	0.00	0.00	0.00	4.05	0.00	

Import from Library...

Import..

Export..

Clear

Apply to..

Close

Acq. Setting - PPP F12 MIX 1_500

Gain

Threshold

Width

FSC

V SSC1

V SSC2

B SSC

Y SSC

R SSC

V447

B531

Y595

R670

R710

R792

100

50

200

100

100

100

1000

1000

1000

1000

1000

1000

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

(1~3000)

Set As Default

Default

QC Gain

Import from File...

Import from Catalog...

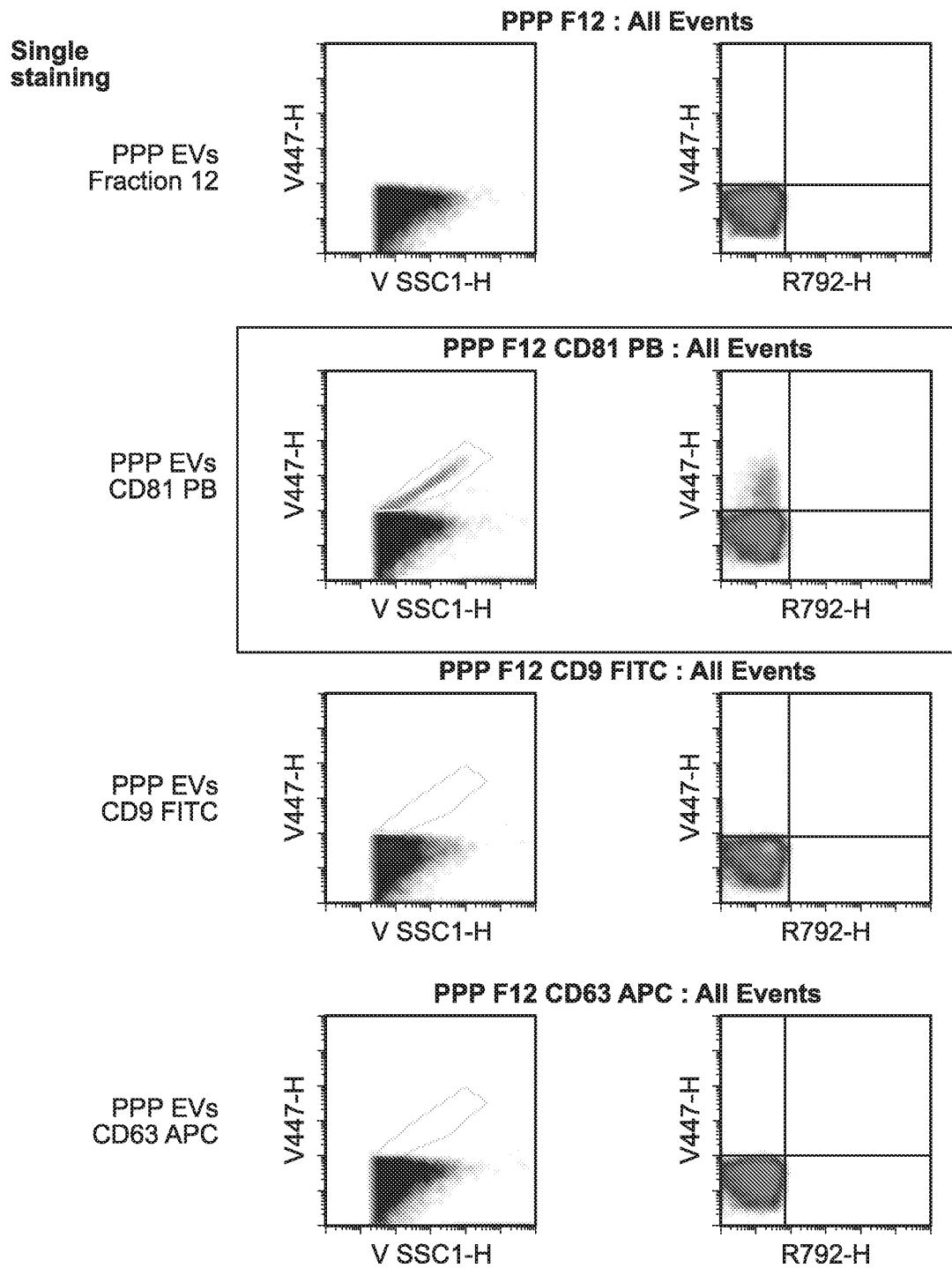
Export to File...

Export to Catalog...

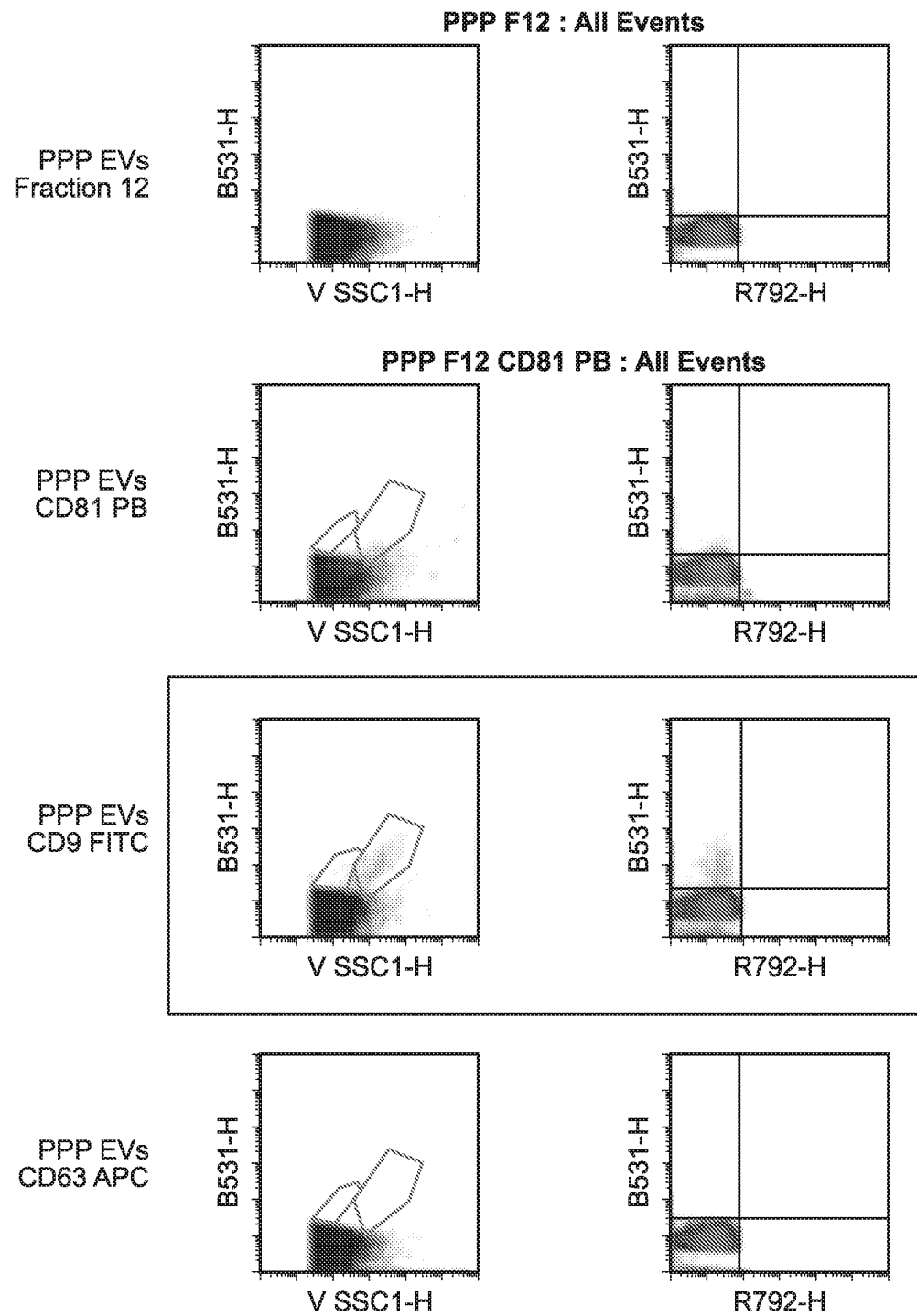
Close

FIG. 30

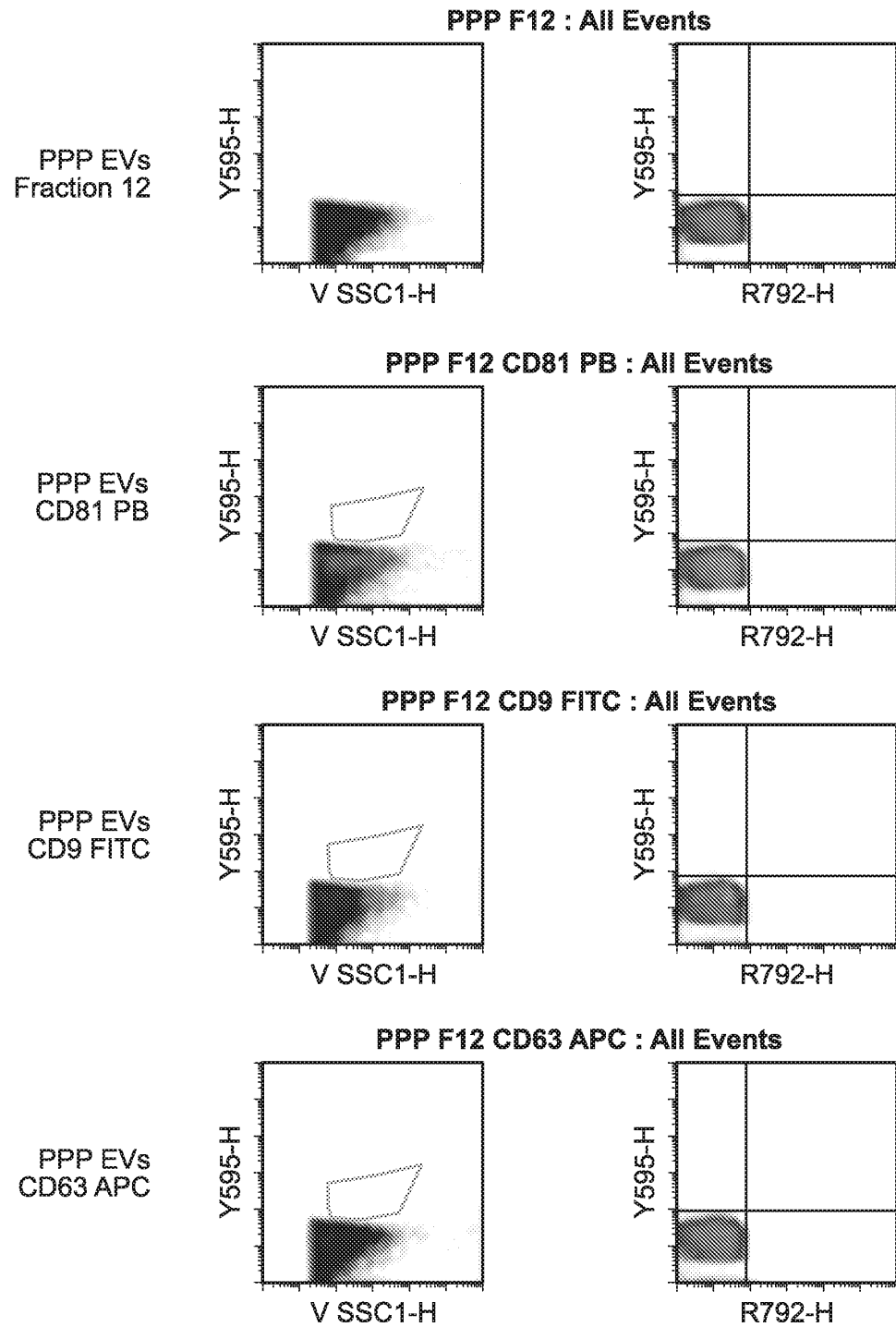
42/127

**FIG. 31**

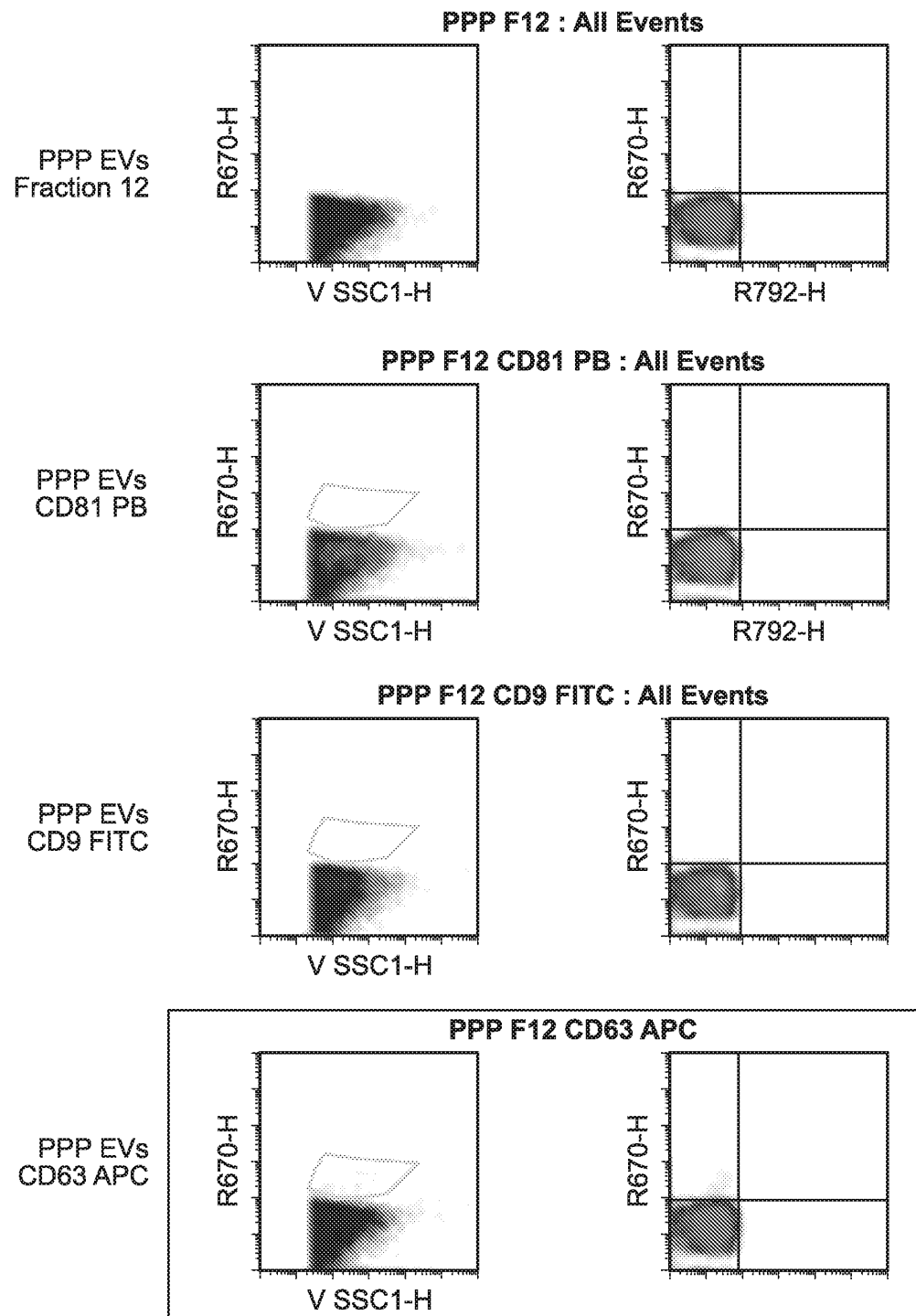
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**FIG. 31 (Cont. 1)**

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**FIG. 31 (Cont. 2)**

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**FIG. 31 (Cont. 3)**

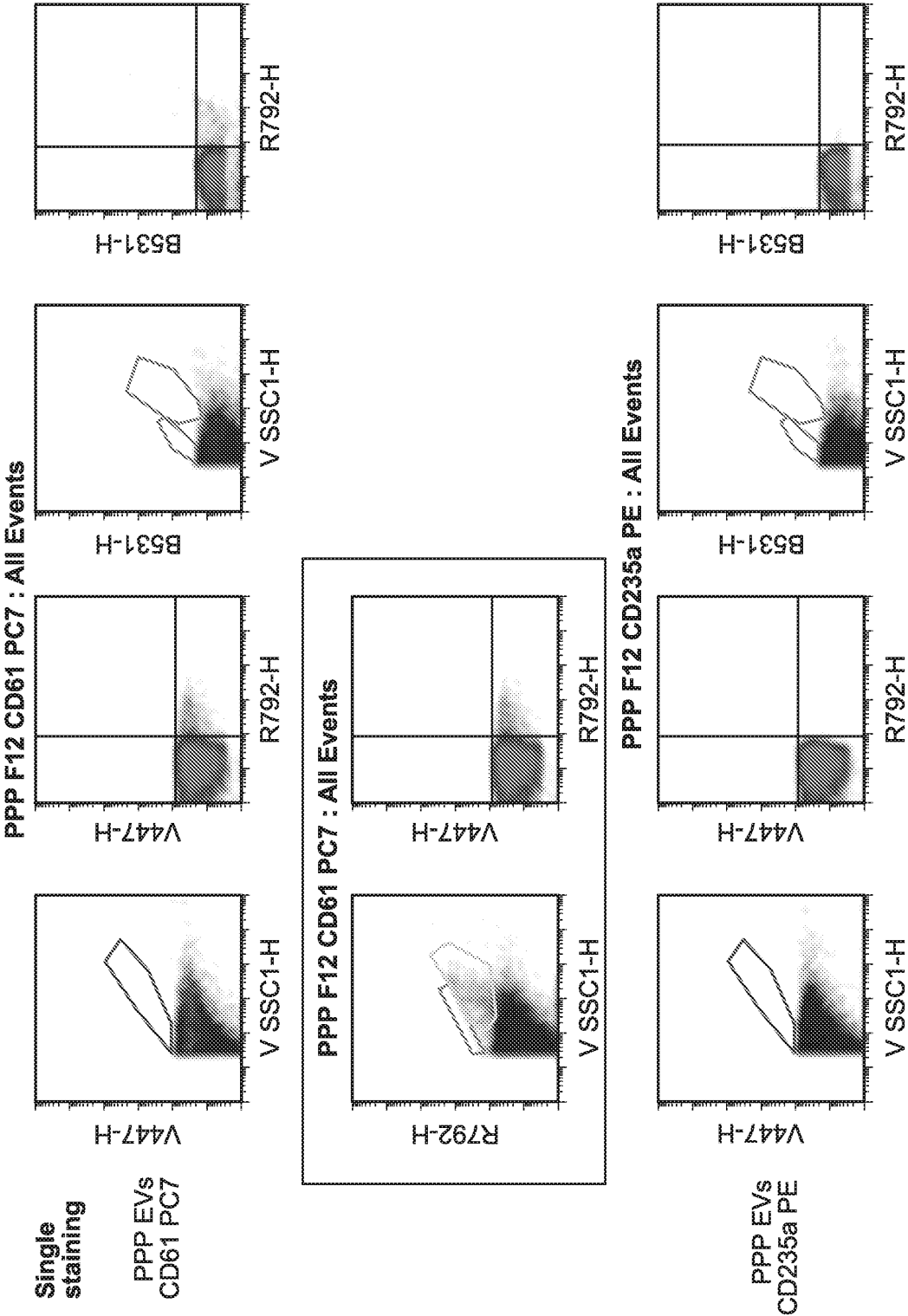
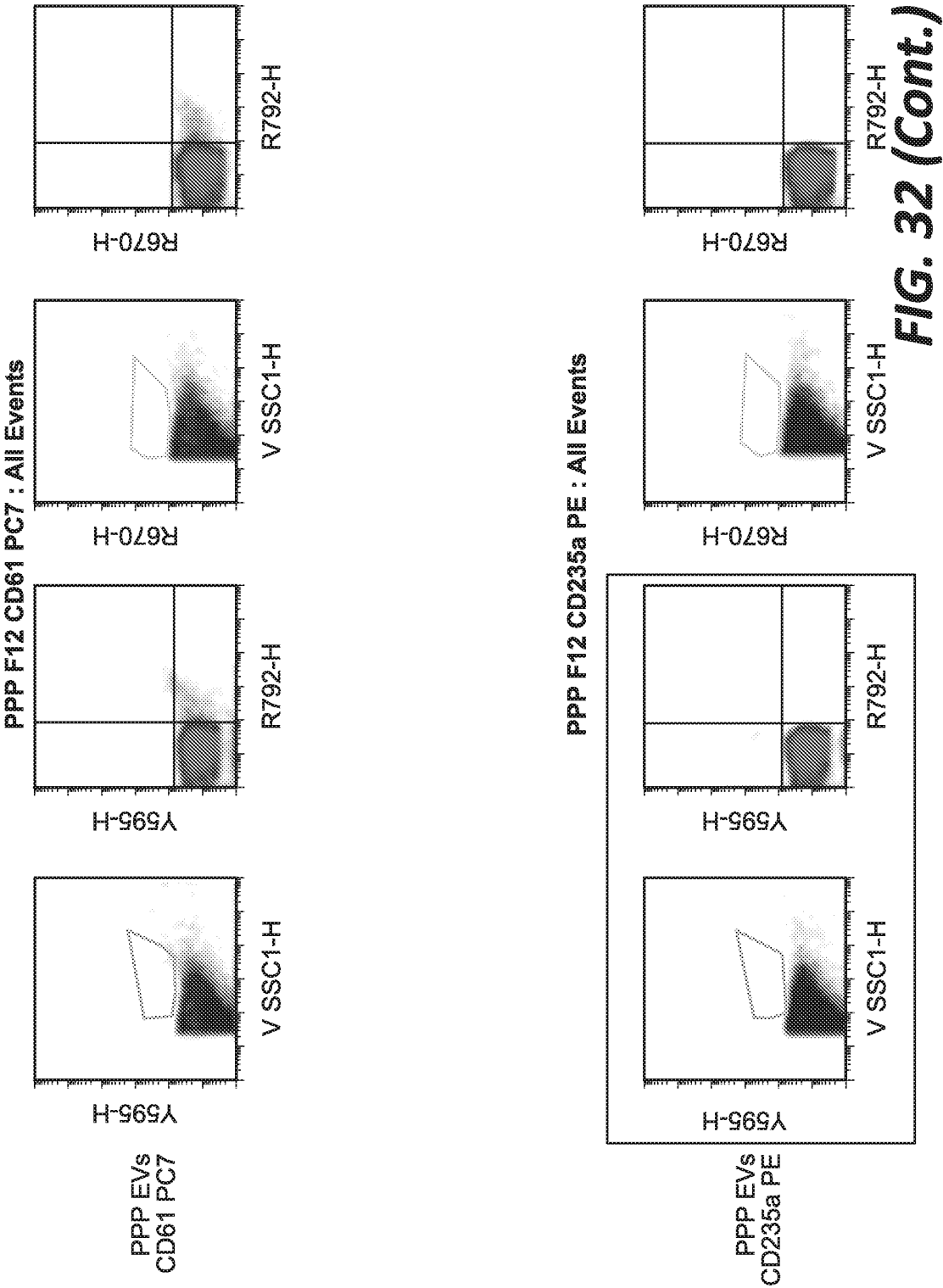


FIG. 32



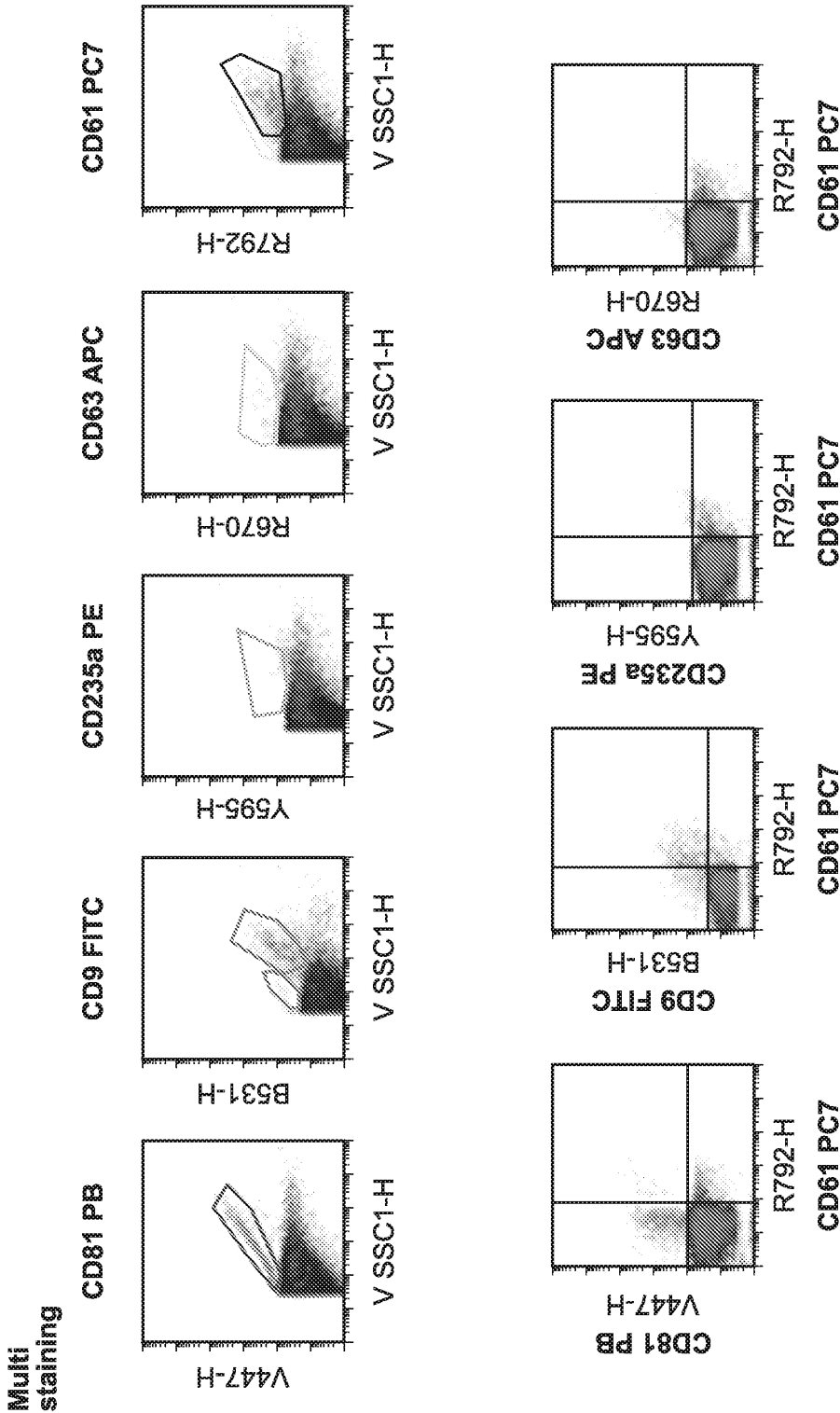
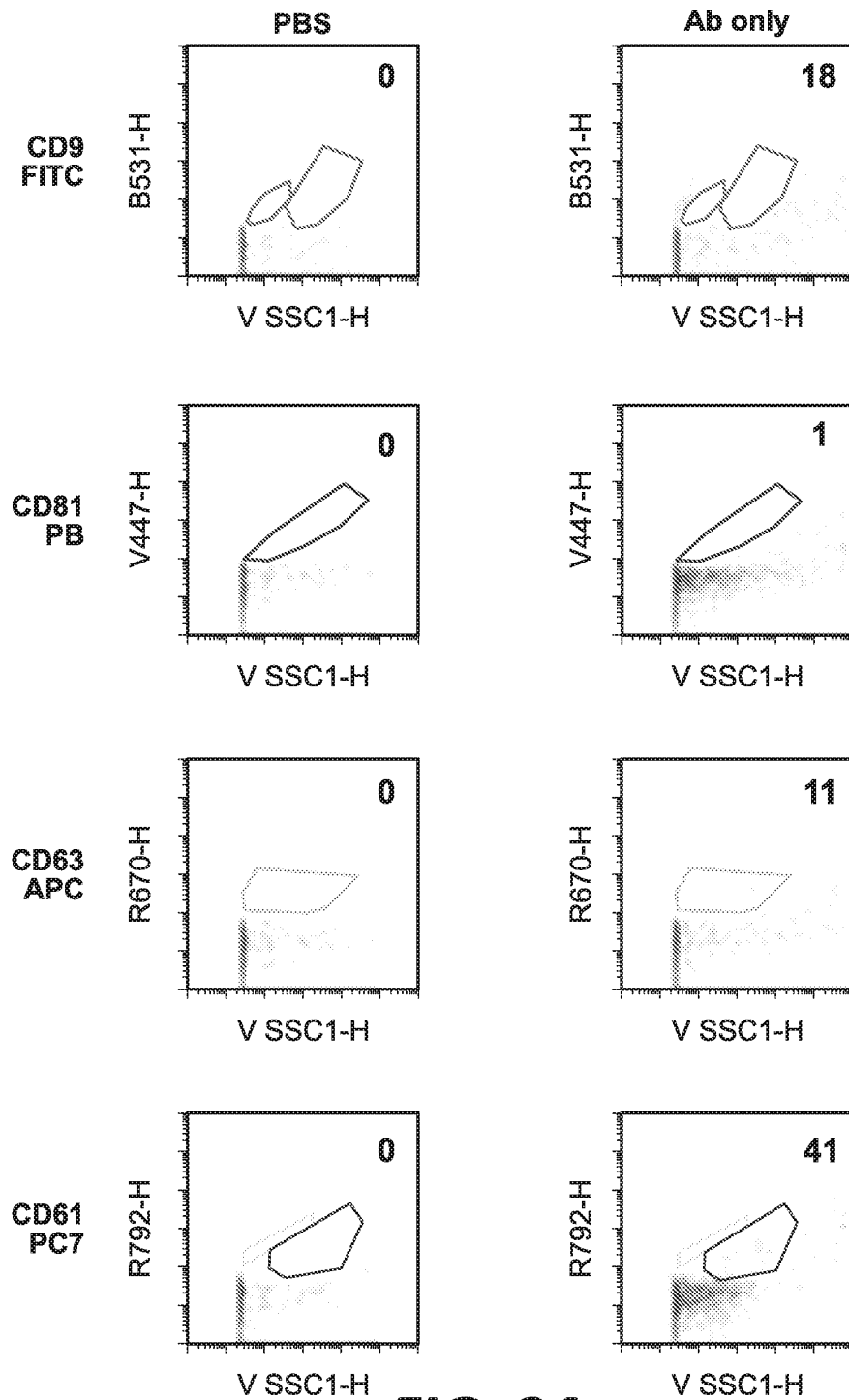


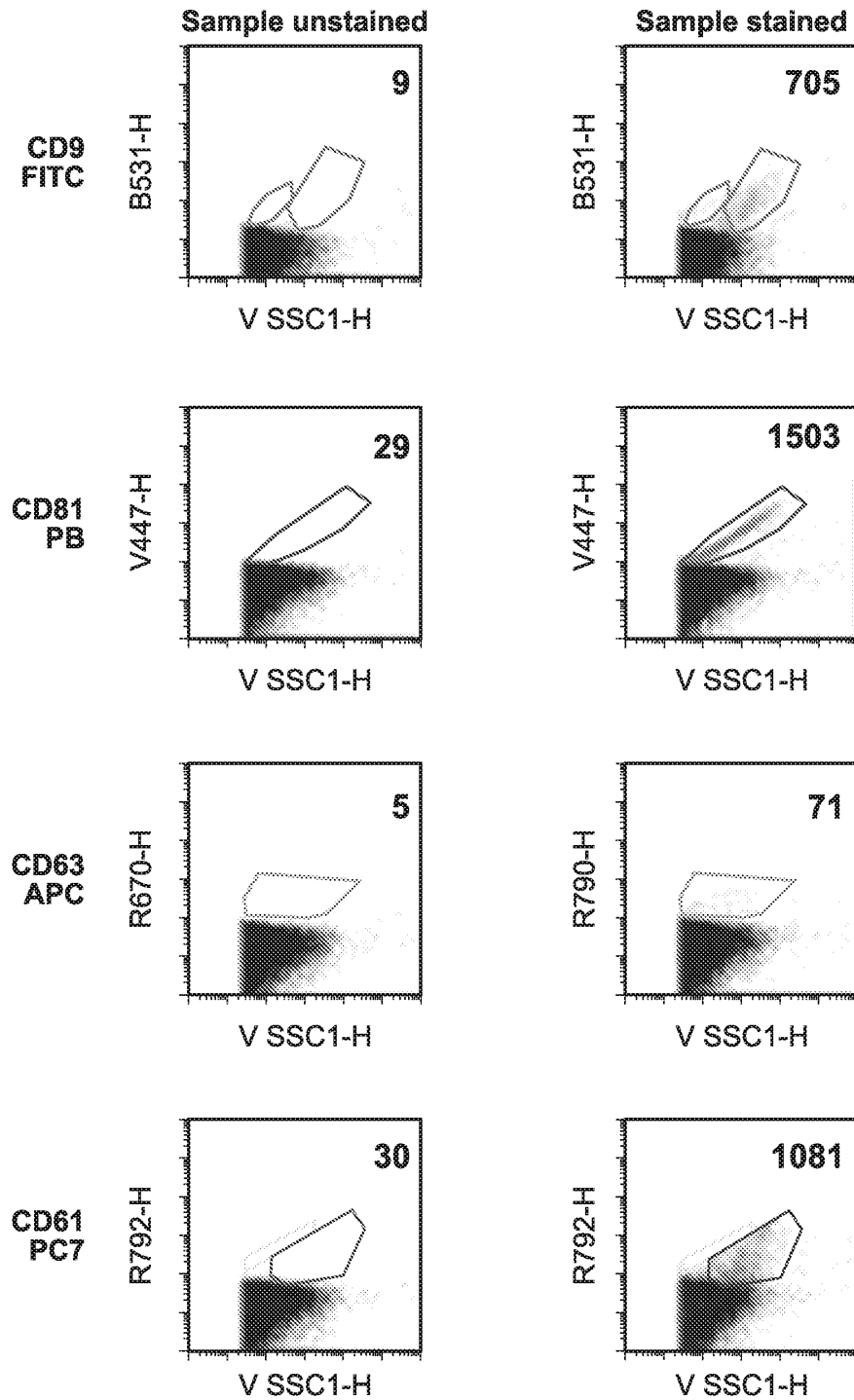
FIG. 33

Antibodies background

**FIG. 34**

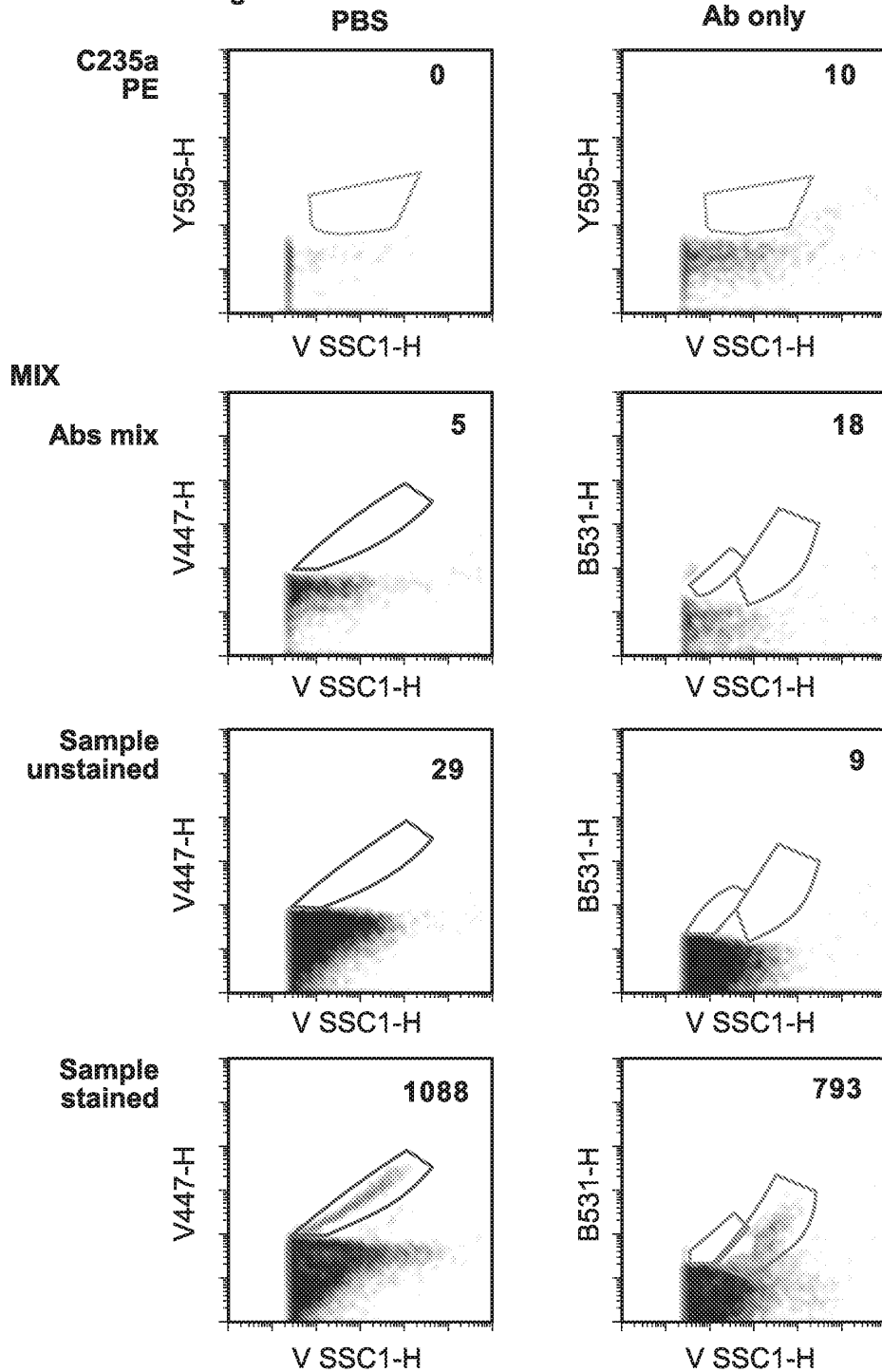
50/127

Antibodies background

**FIG. 34 (Cont.)**

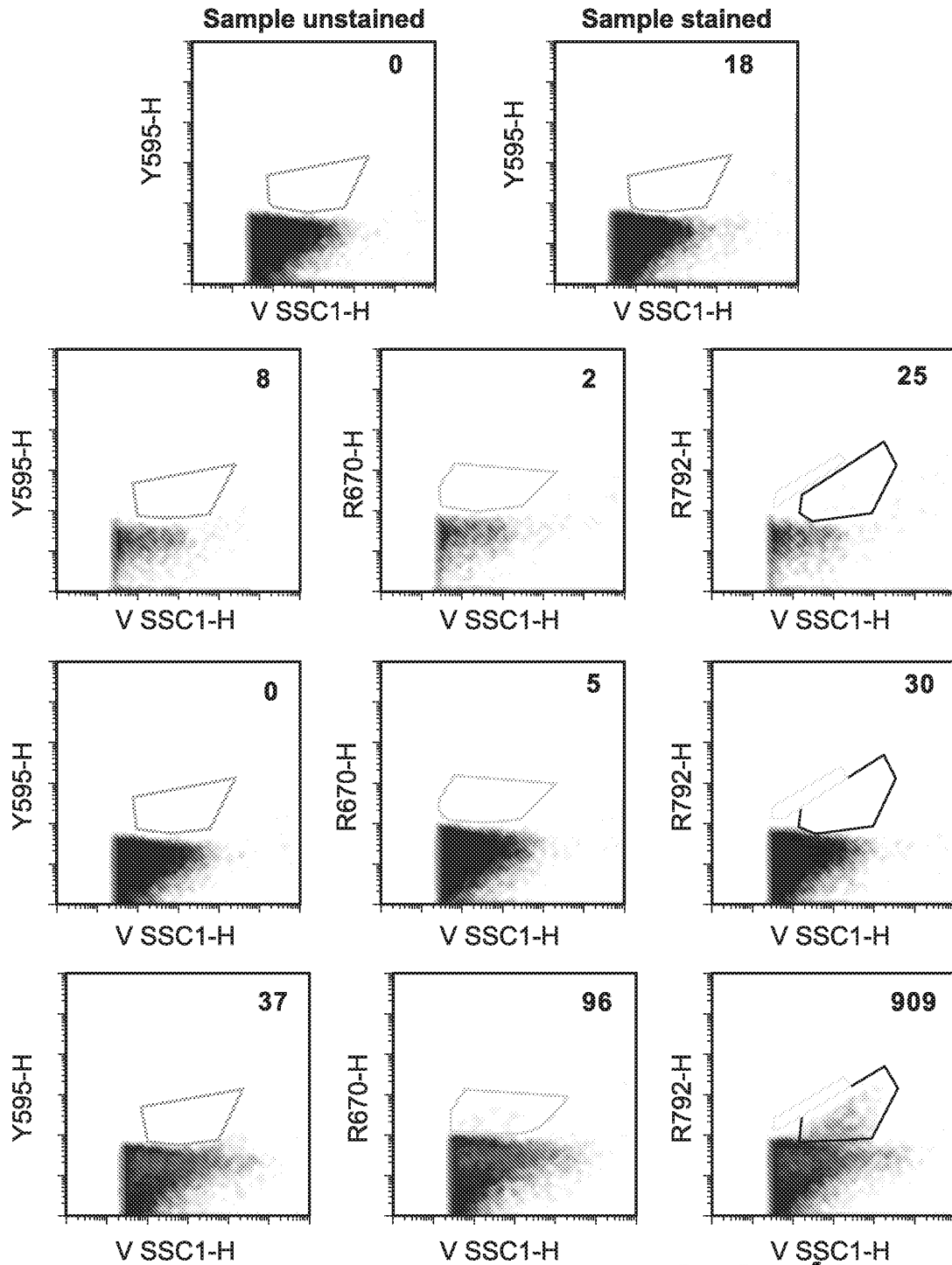
51/127

Antibodies background

**FIG. 35**

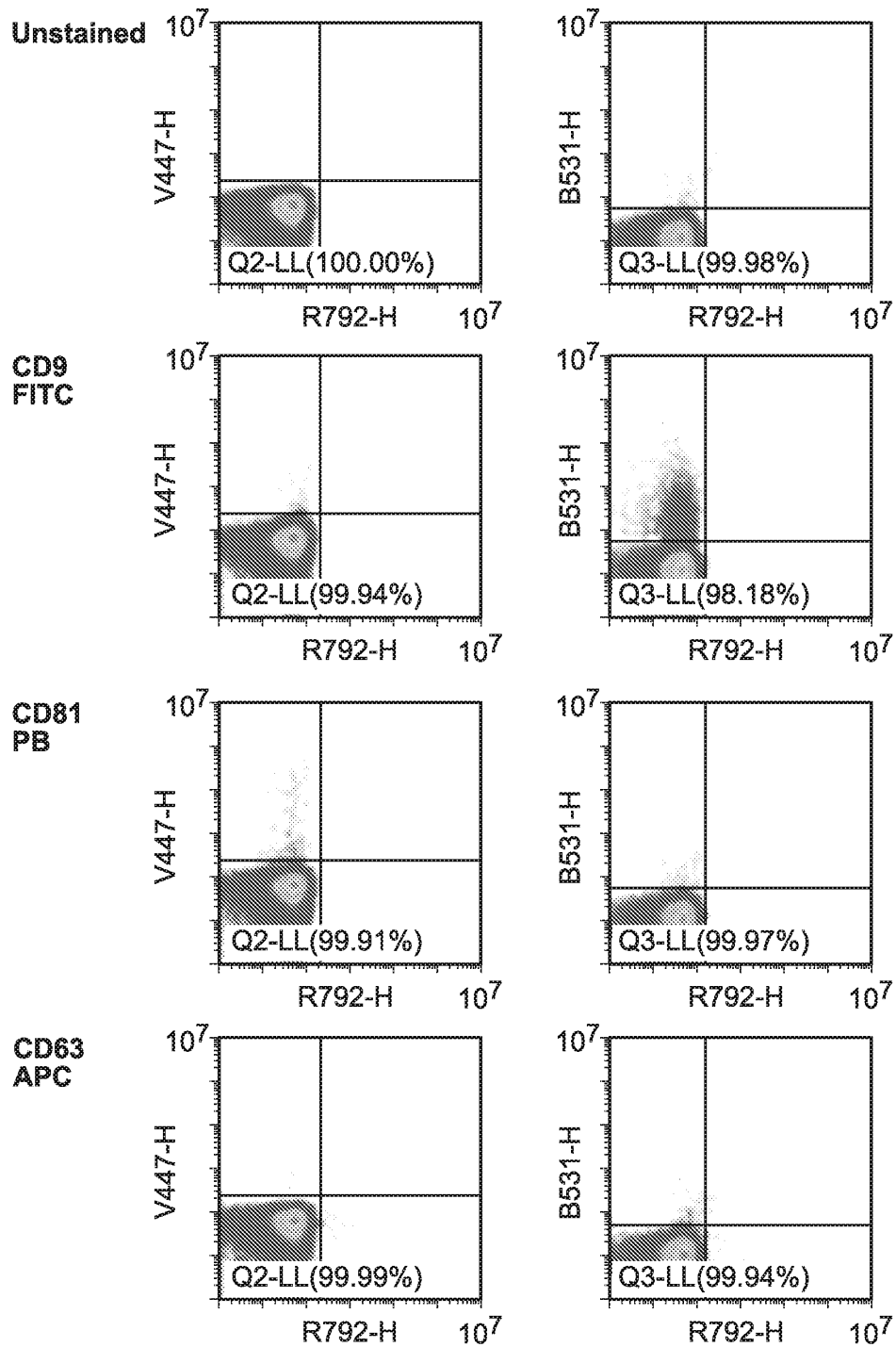
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Antibodies background

**FIG. 35 (Cont.)**

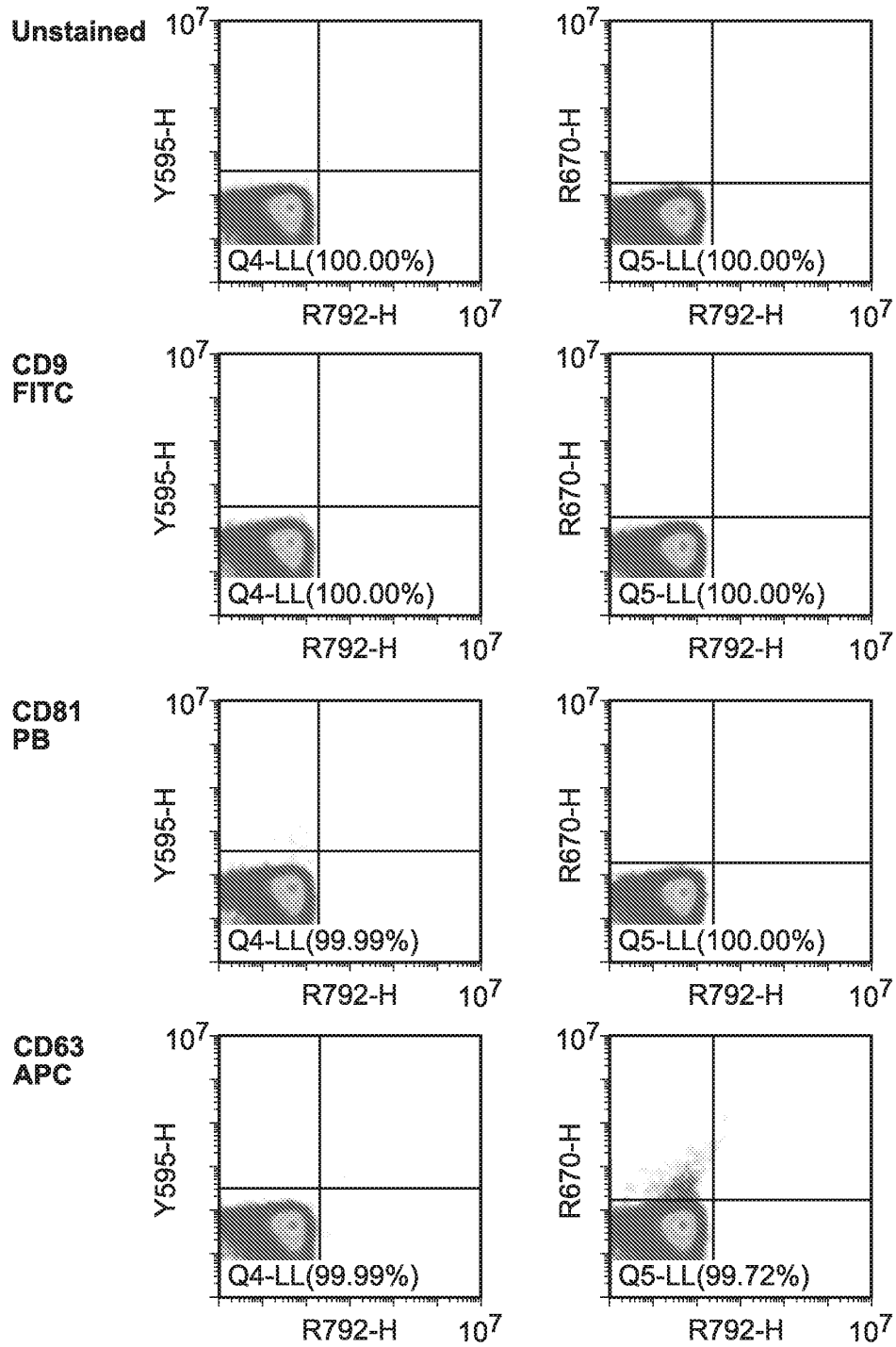
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Izon column 70nm - fraction 6

**FIG. 36**

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Izon column 70nm - fraction 6

**FIG. 36 (Cont.)**

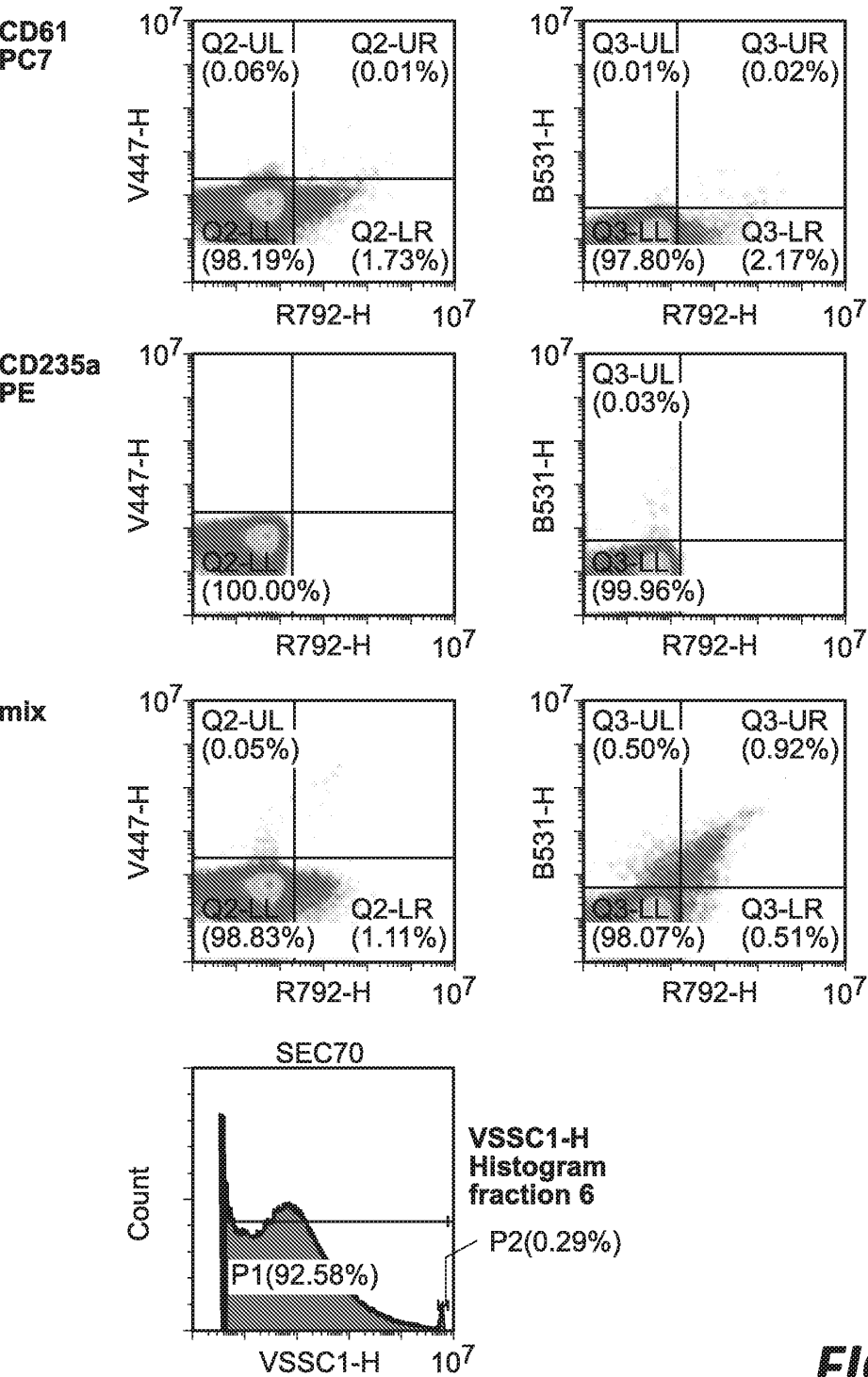


FIG. 37

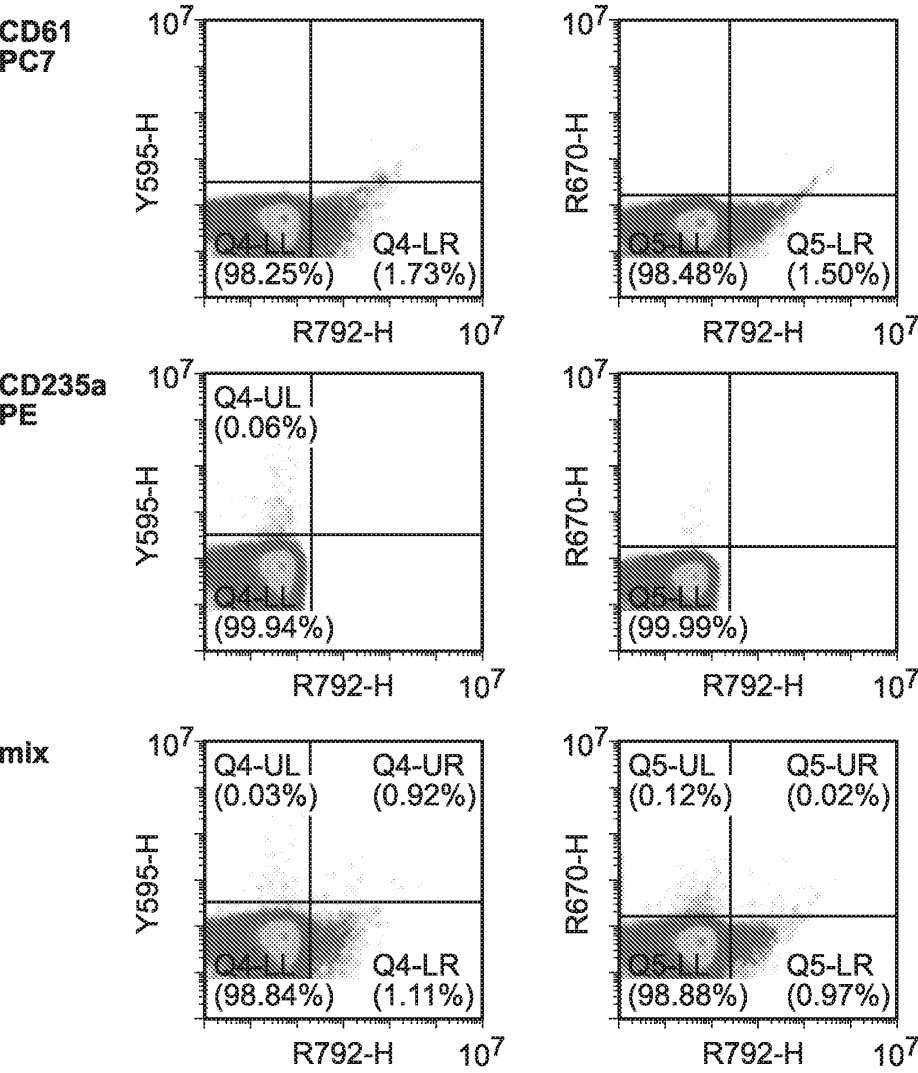


FIG. 37 (Cont.)

Izon column 70nm - fraction 6

Compensation matrix

Compensation Matrix -

☒ Use

☒ Show Auto

☐ Area and Height in Sync

Height ▾

AutoA	Channel	-V447%					
0.00	V447		2.50	0.00	0.00	0.00	0.00
0.00	B531	30.00		0.00	0.00	0.00	2.80
0.00	Y595	0.00	20.00		0.00	0.00	30.00
0.00		0.00	1.33	0.00		0.00	0.00
0.00		0.00	0.00	0.00	0.00		0.00
0.00	R792	0.00	0.32	0.00	5.51	0.00	

Import from Library...

Export...

Clear

Apply to...

Close

Acquisition setting

Acq. Setting -

Gain

Threshold

Width

FSC	458 ▾	(1~3000)
VSSC1	100 ▾	(1~3000)
VSSC2	300 ▾	(1~3000)
BSSC	100 ▾	(1~3000)
YSSC	100 ▾	(1~3000)
RSSC	10 ▾	(1~3000)
V447	1500 ▾	(1~3000)
B531	2000 ▾	(1~3000)
Y595	2500 ▾	(1~3000)
R670	1500 ▾	(1~3000)
R710	1 ▾	(1~3000)
R792	1500 ▾	(1~3000)

Set As Default

QC Gain

Import from File...

Import from Catalog...

Export to File...

Export to Catalog...

Close

Compensation matrix

Compensation Matrix -

☒ Use

☒ Show Auto

☐ Area and Height in Sync

Height ▾

AutoA	Channel	-V447%					
0.00	V447		2.50	0.00	0.00	0.00	0.41
0.00	B531	1.50		0.00	0.00	0.00	2.82
0.00	Y595	0.00	20.00		0.00	0.00	30.45
0.00		0.00	1.33	0.00		0.00	0.44
0.00		0.00	0.00	0.00	0.00		0.00
0.00	R792	0.00	0.32	0.00	0.00	0.00	

Import from Library...

Export...

Clear

Apply to...

Close

FIG. 38

Izon column 70nm - fraction 6

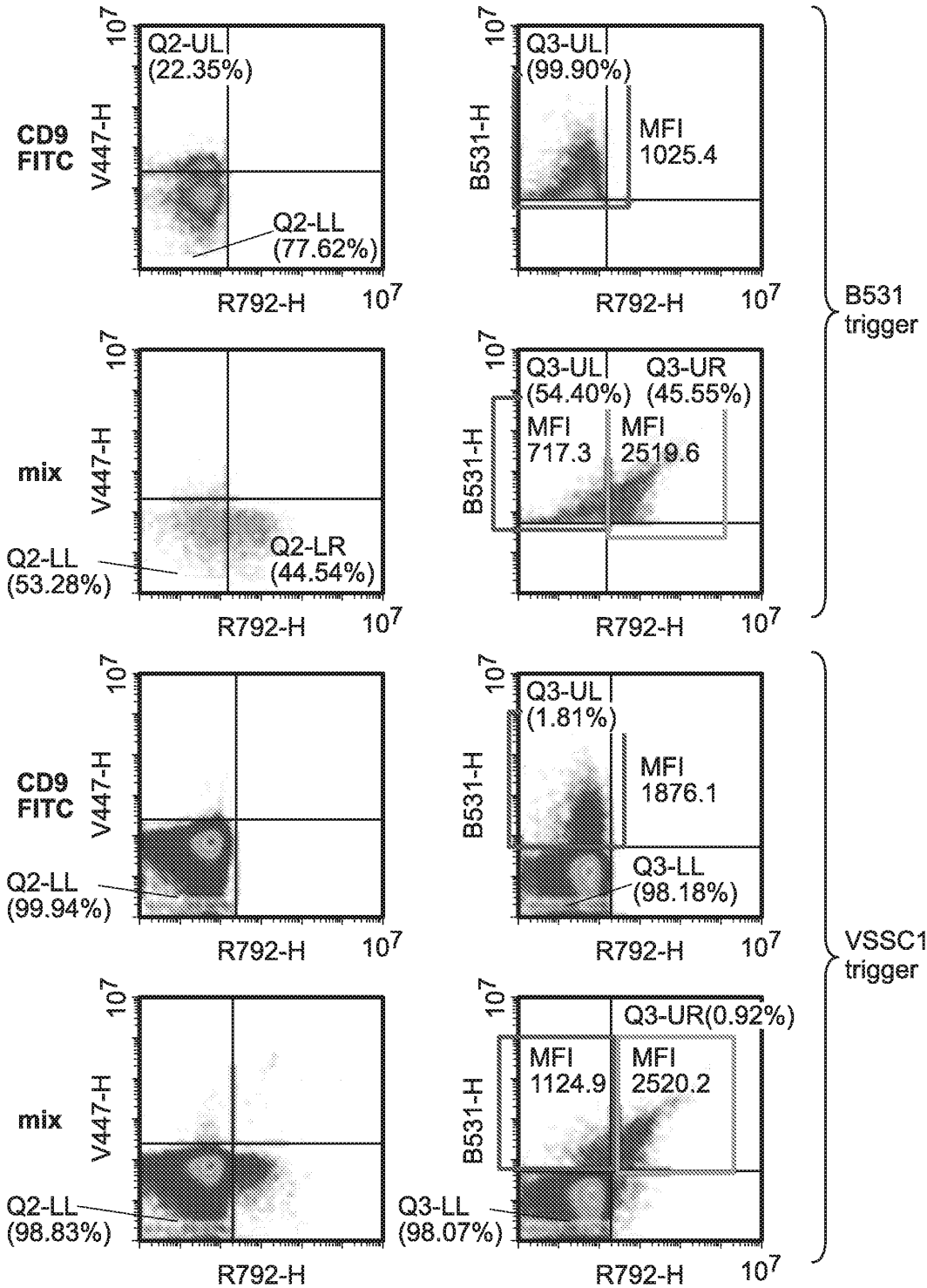
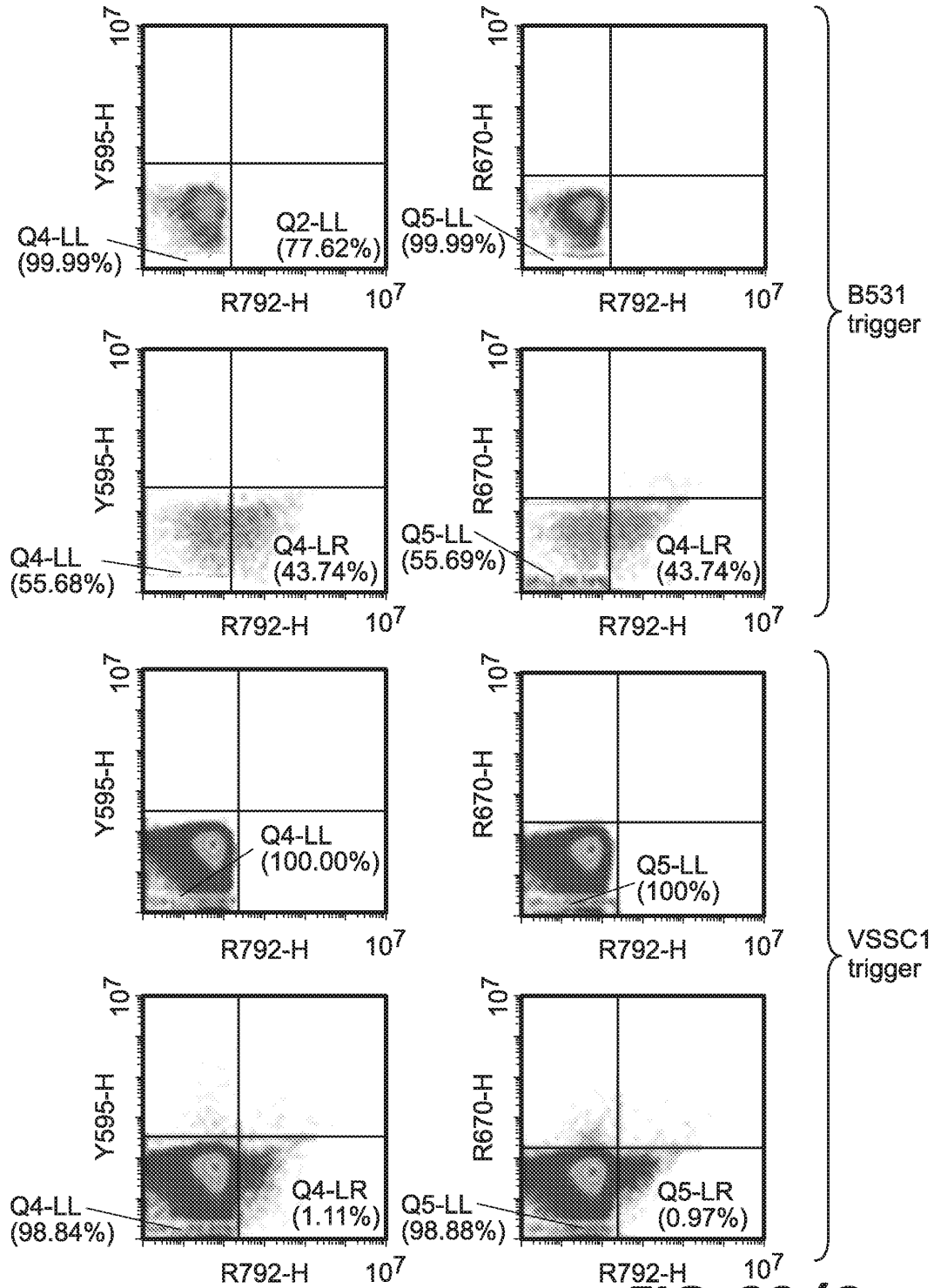


FIG. 39

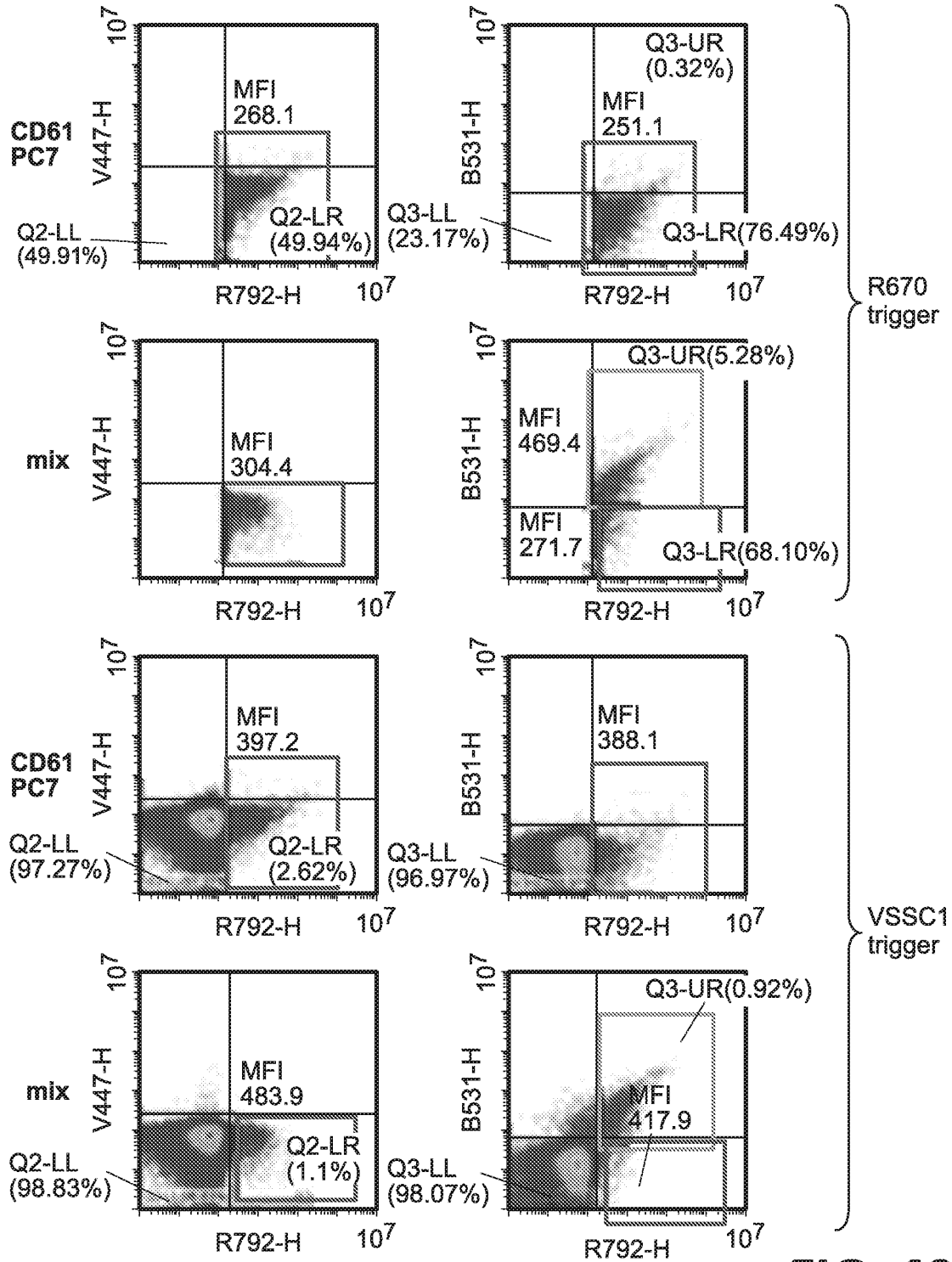
59/127

Izon column 70nm - fraction 6

**FIG. 39 (Cont.)**

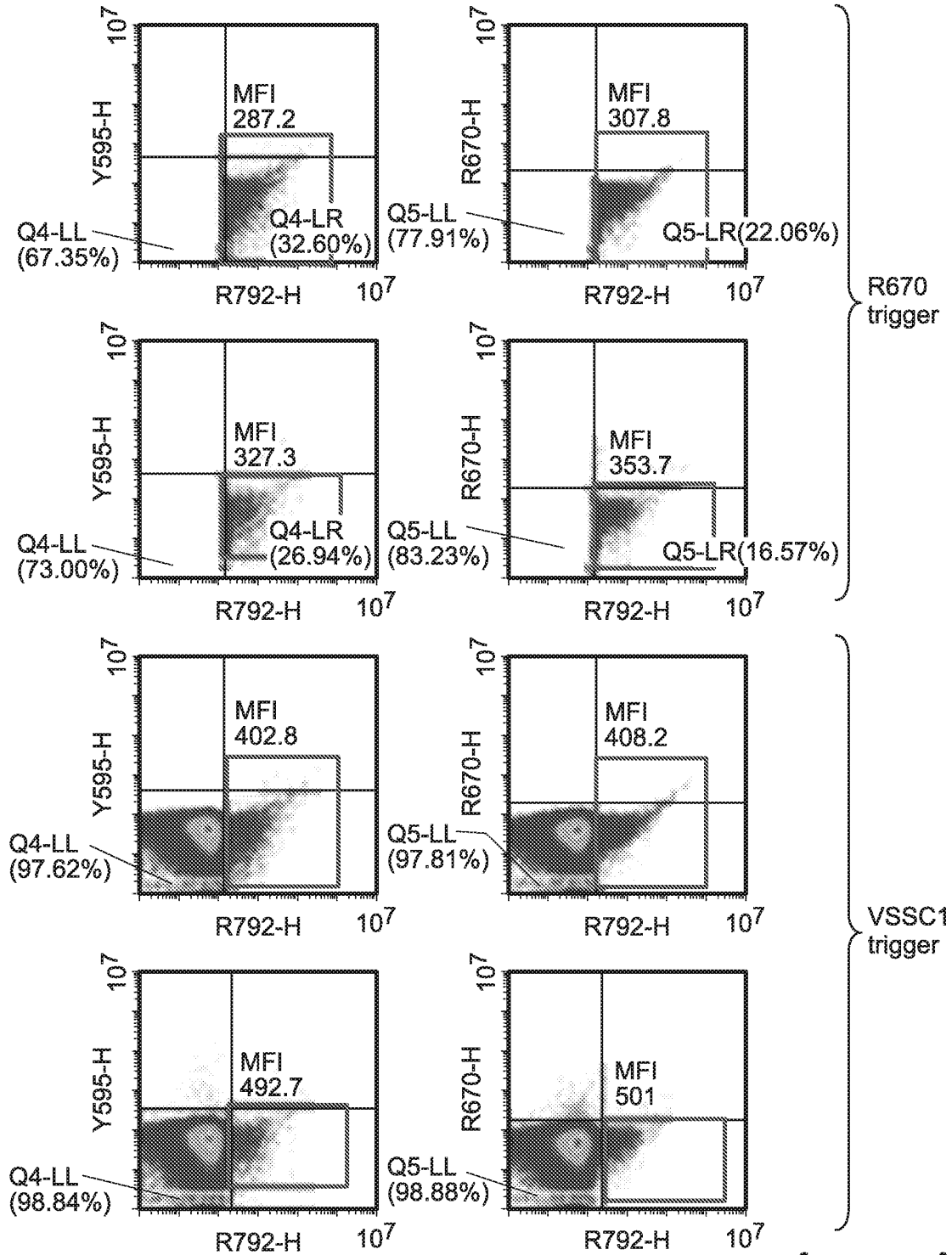
60/127

Izon column 70nm - fraction 6

**FIG. 40**

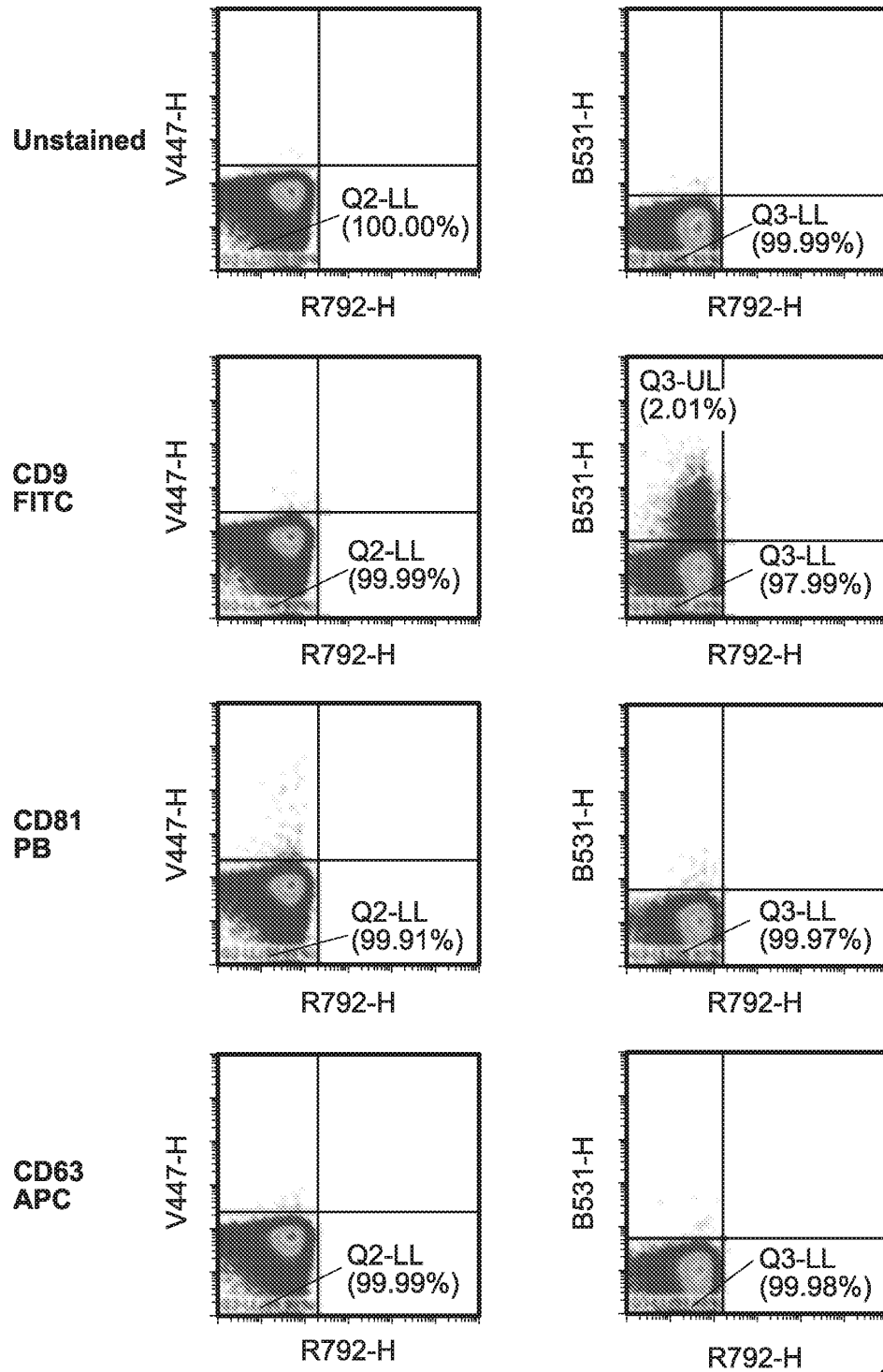
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Izon column 70nm - fraction 6

**FIG. 40 (Cont.)**

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Izon column 35nm - fraction 6

**FIG. 41**

SUBSTITUTE SHEET (RULE 26)

Izon column 35nm - fraction 6

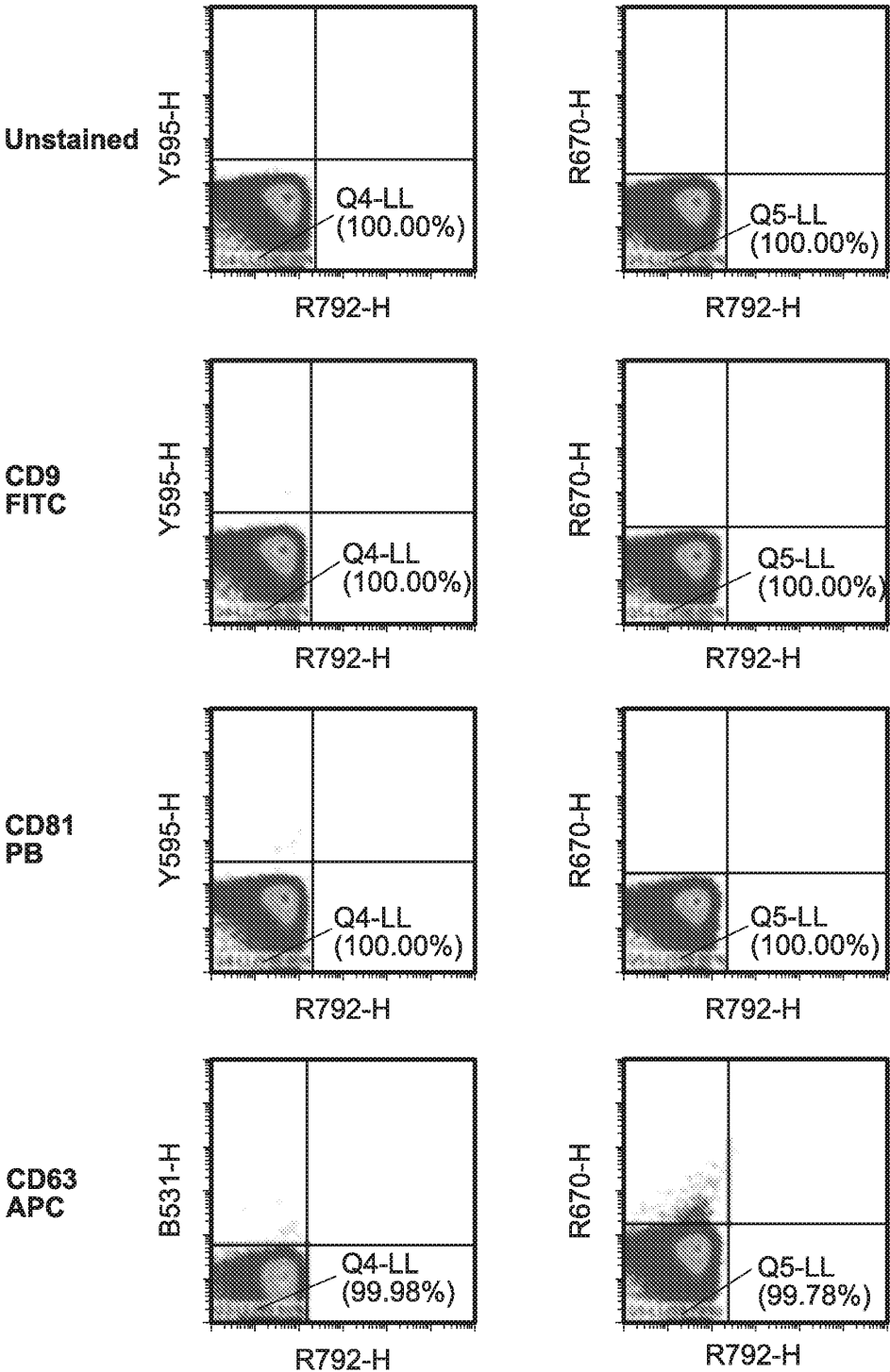


FIG. 41 (Cont.)

Izon column 35nm - fraction 6

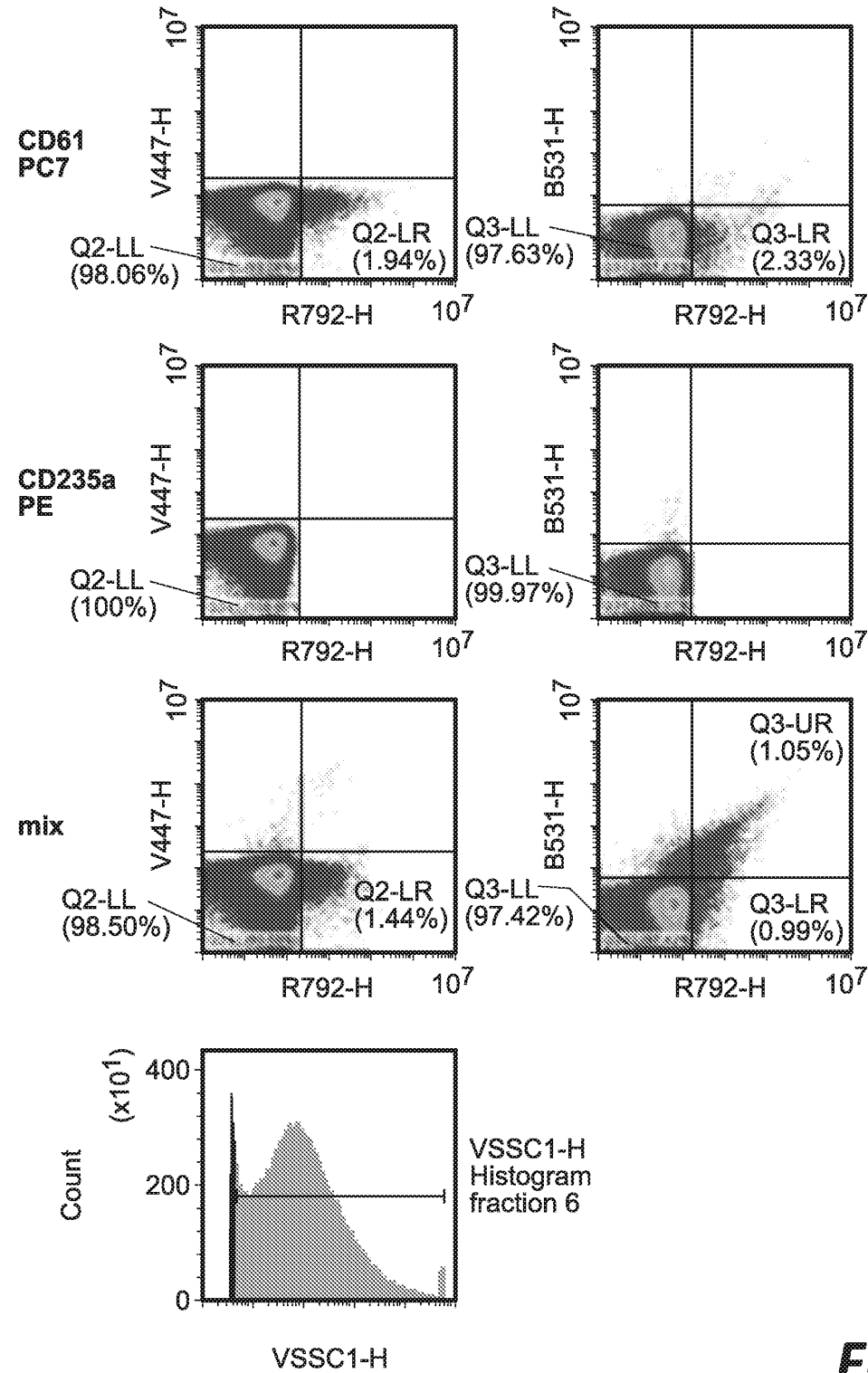
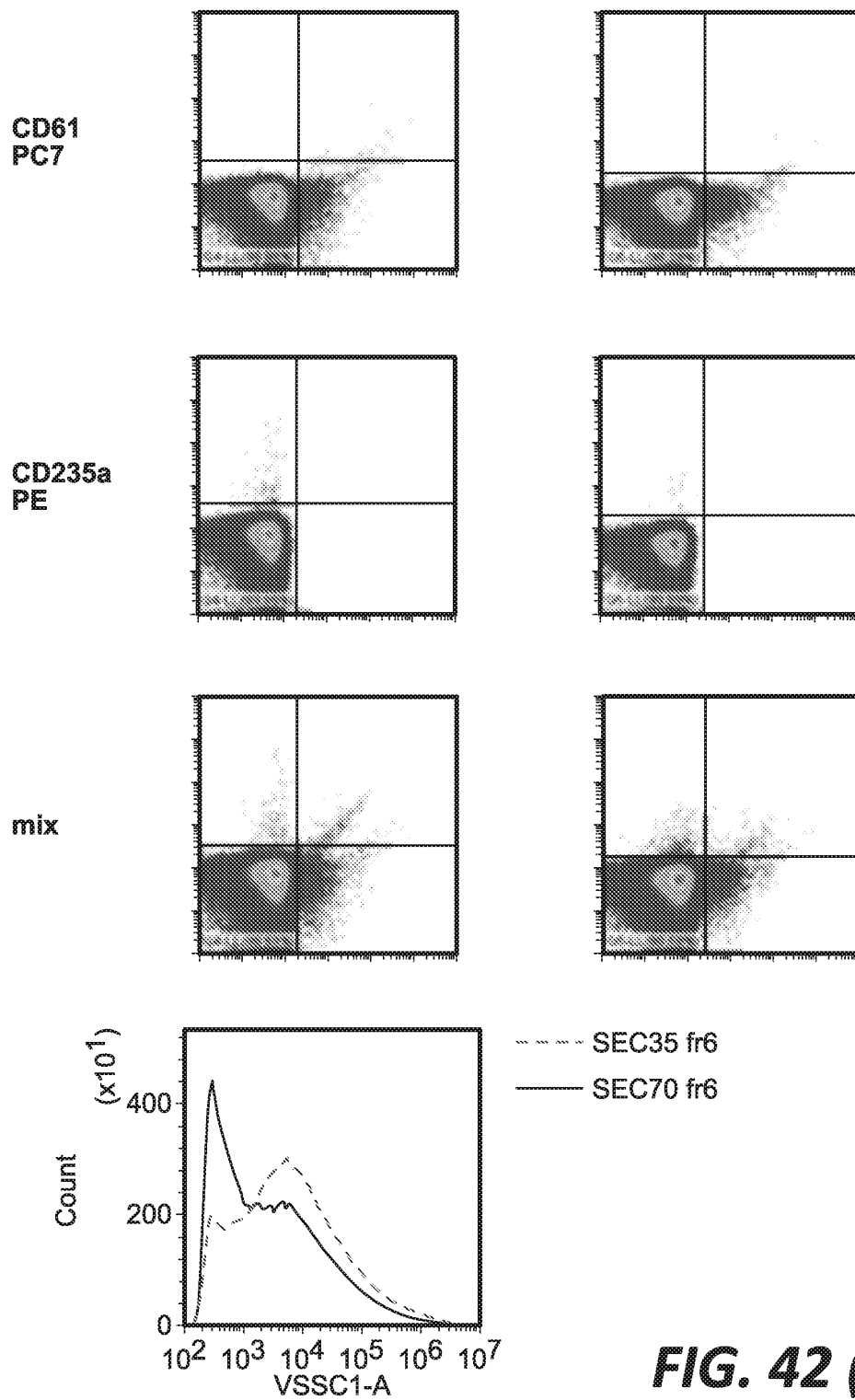


FIG. 42

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Izon column 35nm - fraction 6

**FIG. 42 (Cont.)**

Izon column 70nm - fraction 6

Compensation matrix

Compensation Matrix -

☒ Use

☒ Show Auto

☐ Area and Height in Sync

Height ▾

AutoA	Channel	-V447%					
0.00			2.50	0.00	0.00	0.00	
0.00		30.00		0.00	0.00	0.00	
0.00		0.00	20.00		0.00	0.00	
0.00		0.00	1.33	0.00		0.00	
0.00		0.00	0.00	0.00	0.00		0.00
0.00		0.00	0.32	0.00		0.00	

Import from Library...

Export...

Clear

Apply to...

Close

Acquisition setting

Acq. Setting -

Gain

Threshold

Width

FSC

458 ▾

(1~3000)

VSSC1

100 ▾

(1~3000)

VSSC2

300 ▾

(1~3000)

BSSC

100 ▾

(1~3000)

YSSC

100 ▾

(1~3000)

RSSC

10 ▾

(1~3000)

V447

1500 ▾

(1~3000)

B531

2000 ▾

(1~3000)

Y595

2500 ▾

(1~3000)

R670

1500 ▾

(1~3000)

R710

1 ▾

(1~3000)

R792

1500 ▾

(1~3000)

Set As Default

Default

QC Gain

Import from File...

Import from Catalog...

Export to File...

Export to Catalog...

Close

Compensation matrix

Compensation Matrix -

☒ Use

☒ Show Auto

☐ Area and Height in Sync

Height ▾

0.00	V447		2.50	0.00	0.00	0.00	
0.00	B531	1.50		0.00	0.00	0.00	
0.00	Y595	0.00	20.00		0.00	0.00	
0.00		0.00	1.33	0.00		0.00	
0.00		0.00	0.00	0.00	0.00		0.00
0.00	R792	0.00	0.32	0.00	0.00	0.00	

Import from Library...

Export...

Clear

Apply to...

Close

FIG. 43

SUBSTITUTE SHEET (RULE 26)

Izon column 35nm - fraction 6

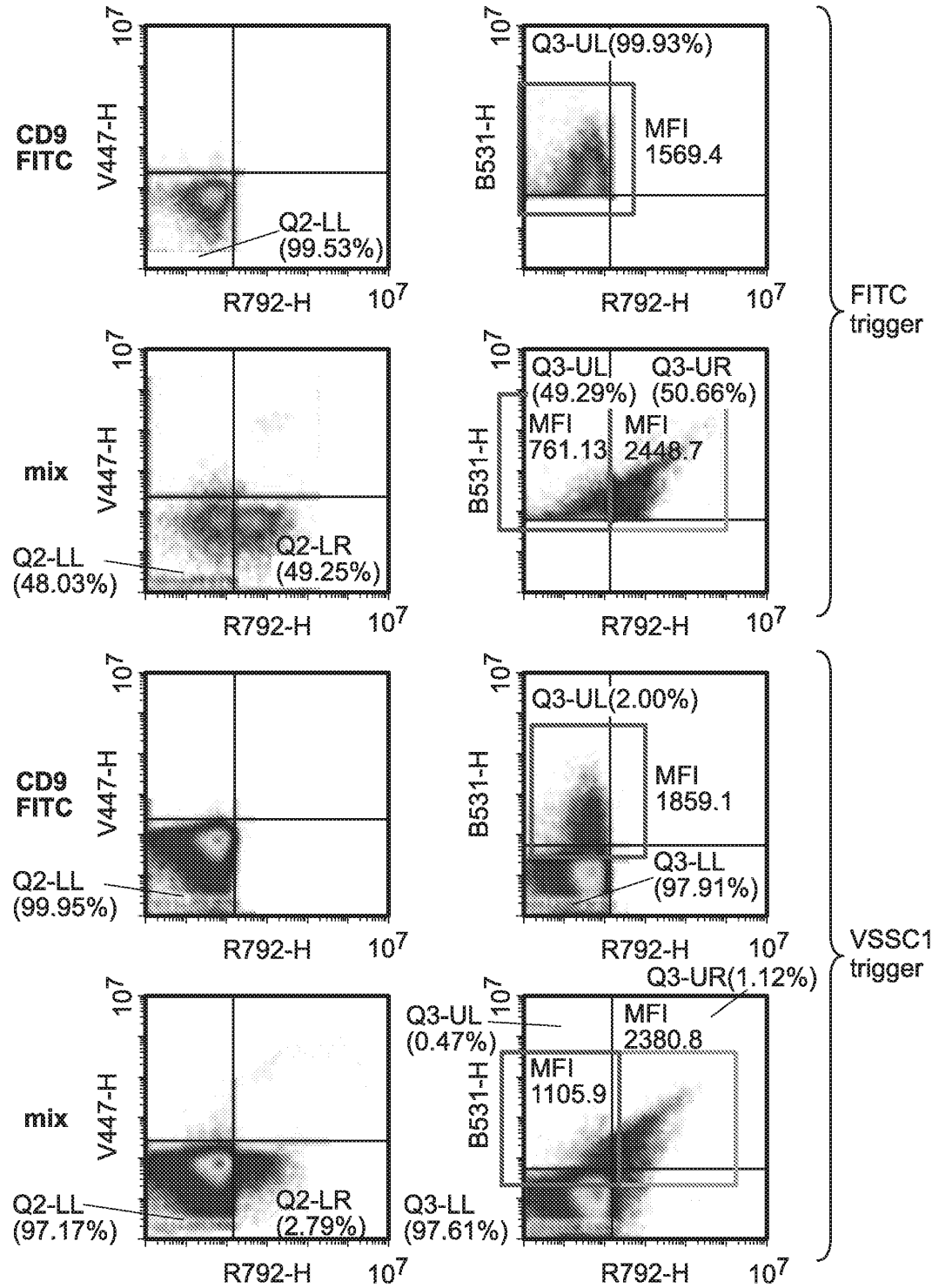
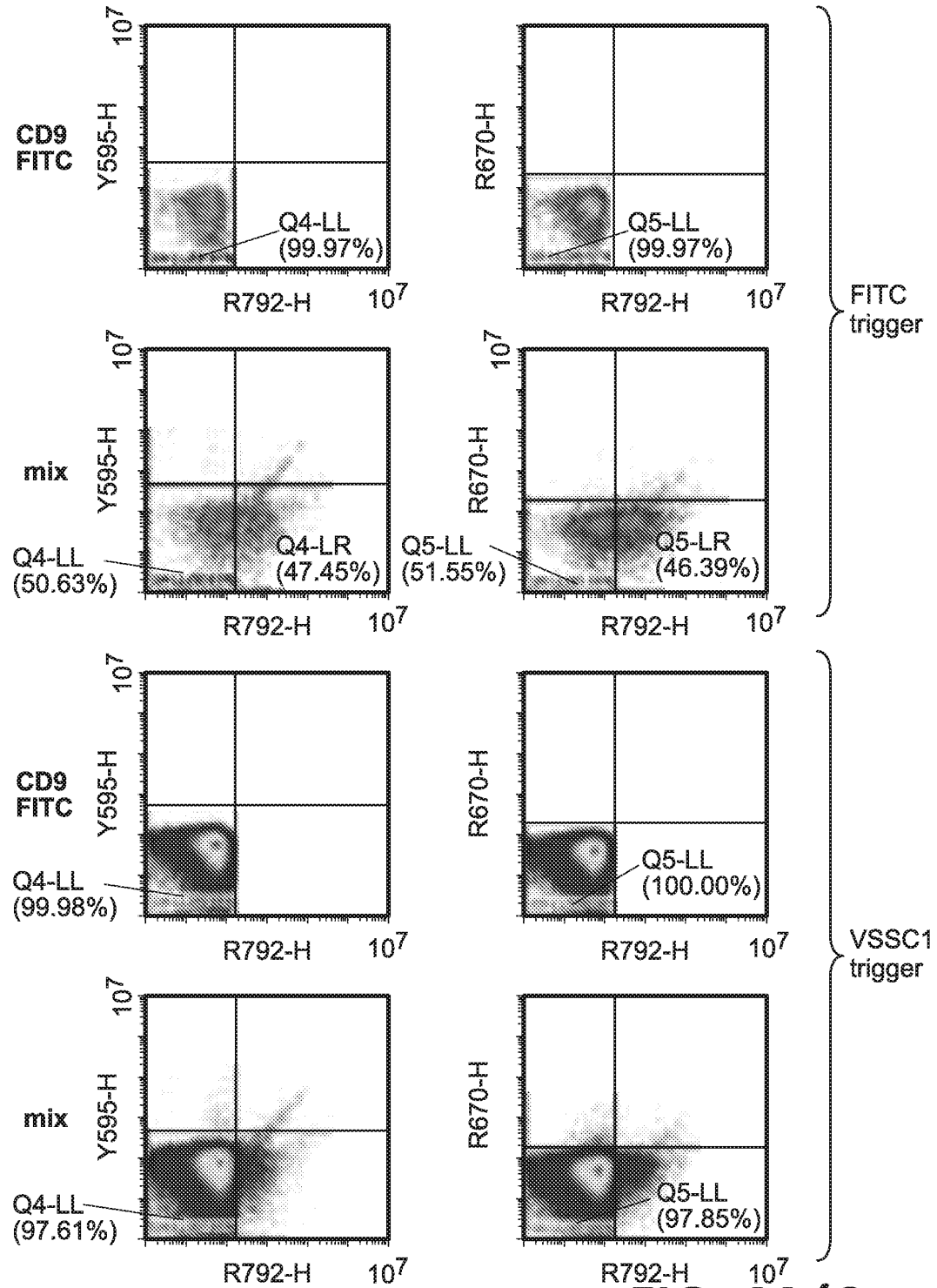


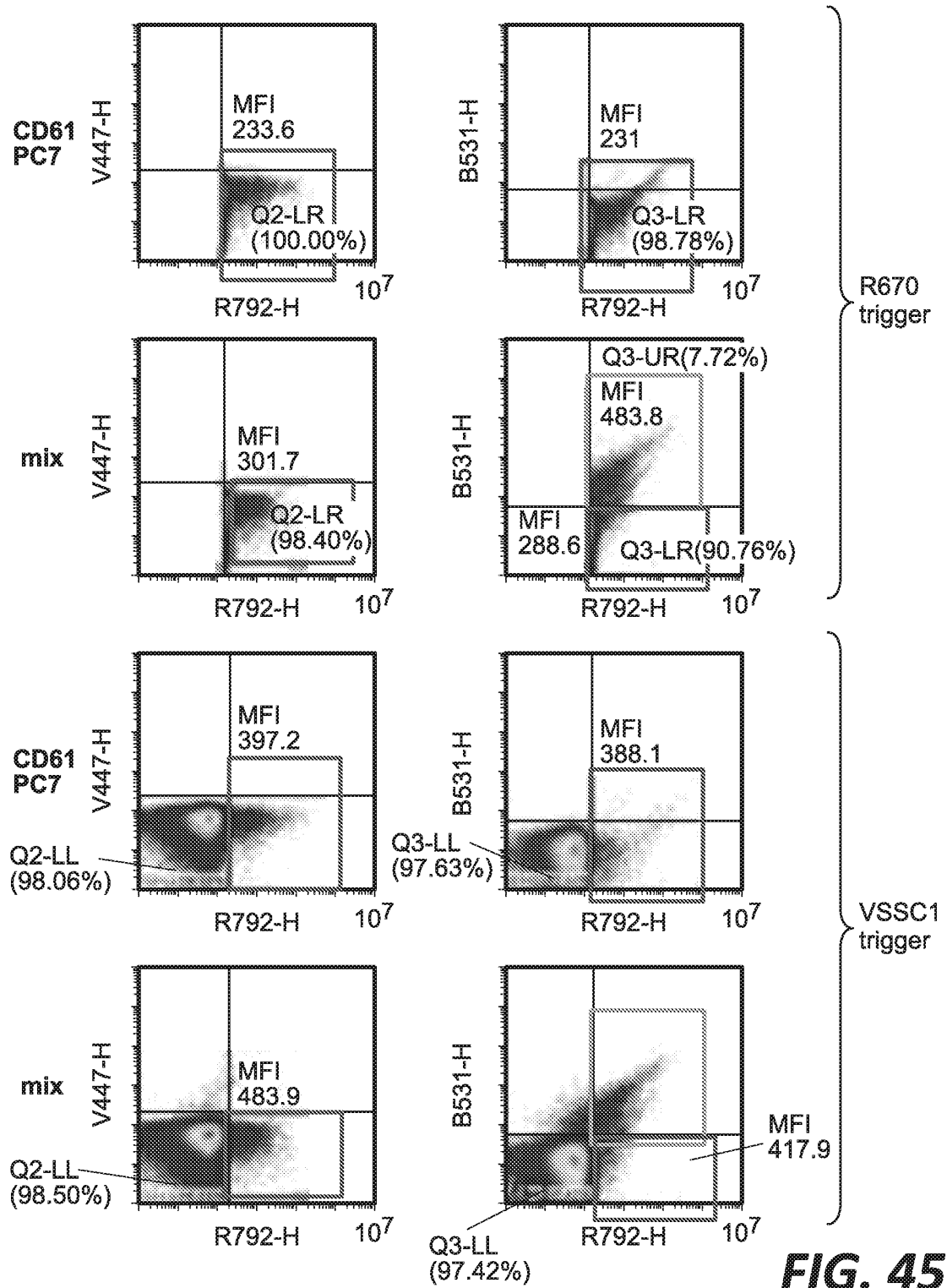
FIG. 44

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Izon column 35nm - fraction 6

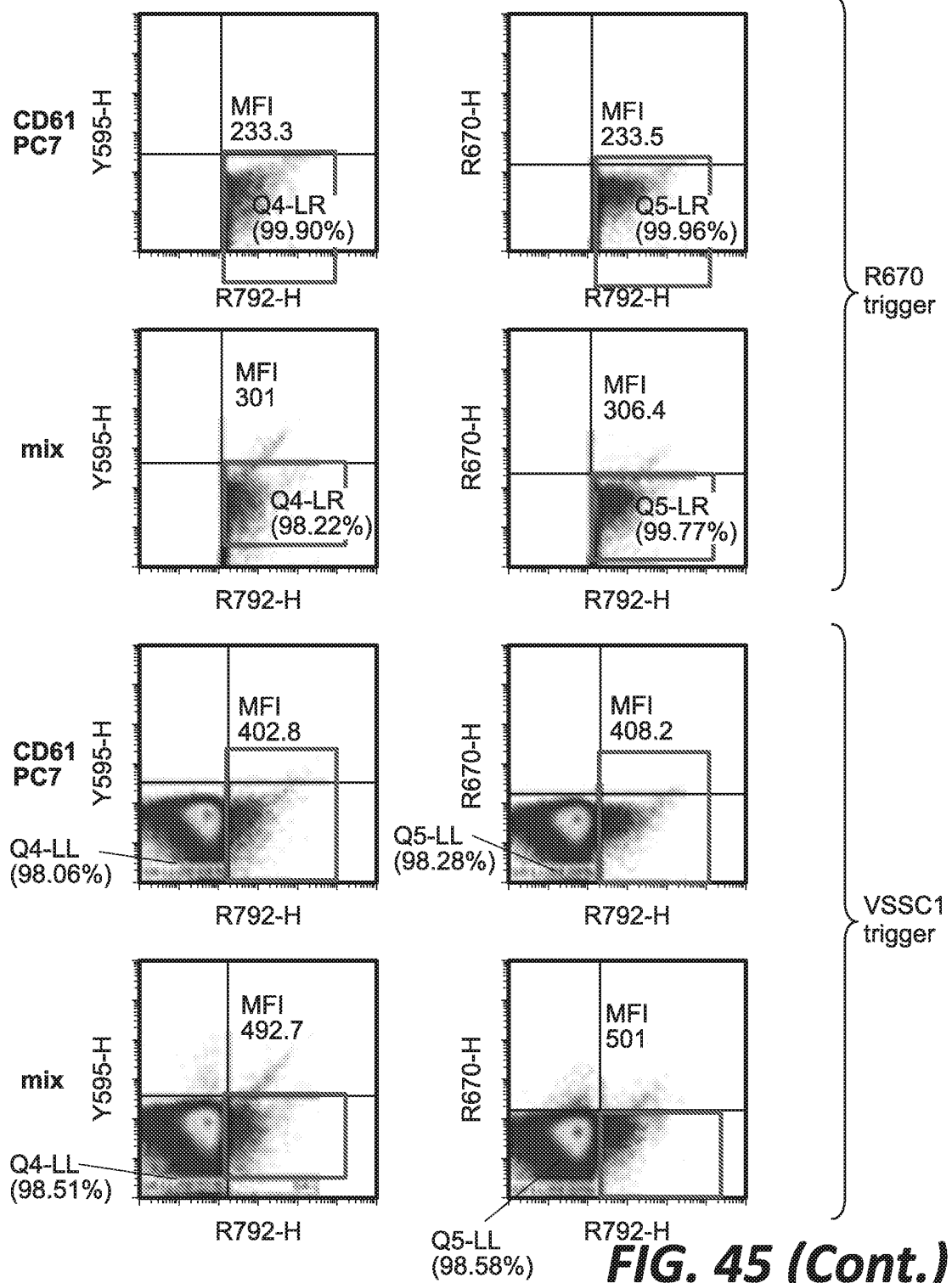
**FIG. 44 (Cont.)**

Izon column 35nm - fraction 6

**FIG. 45**

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Izon column 35nm - fraction 6



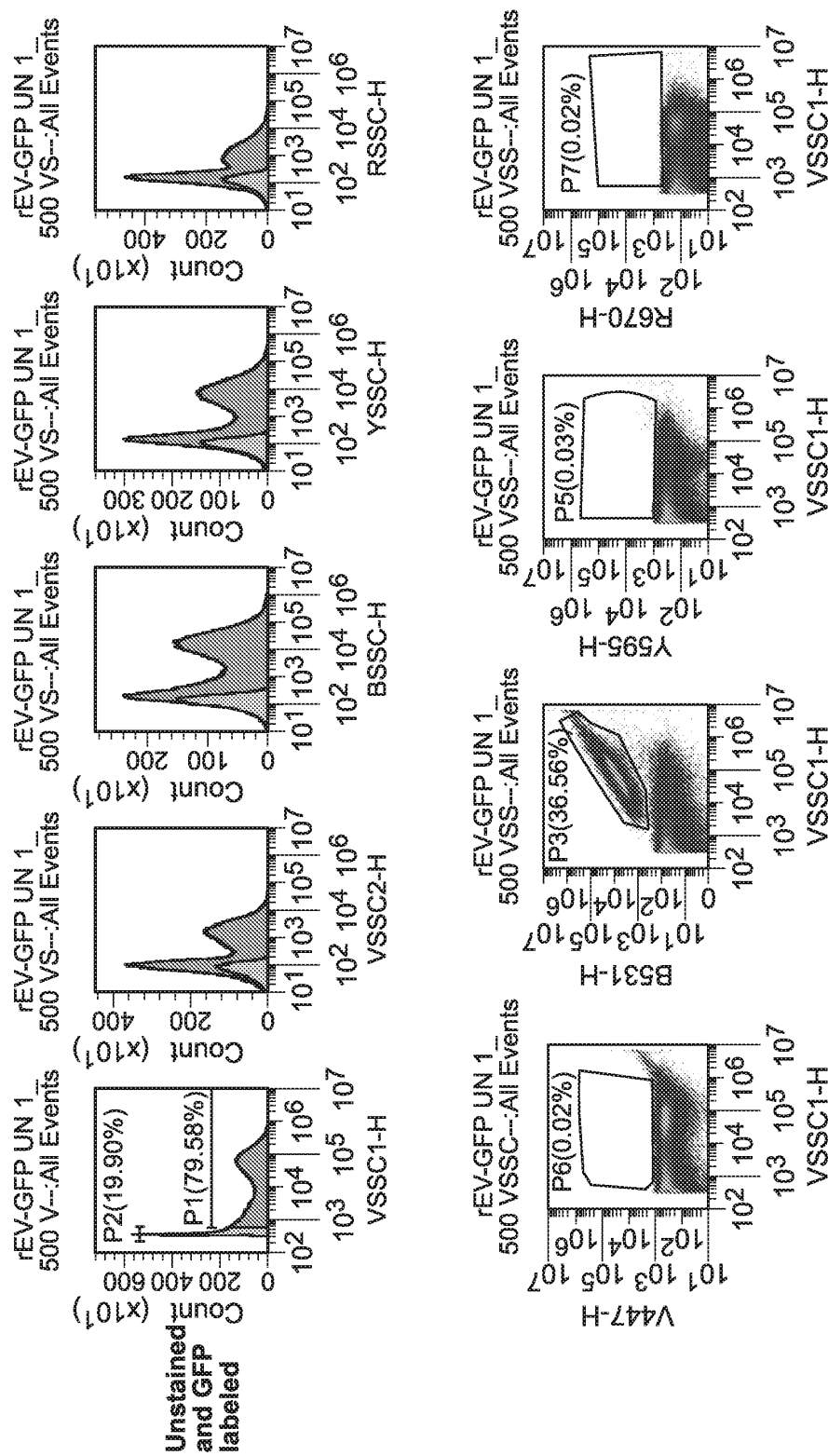


FIG. 46

Acq. Setting - rEV-GFP UN 1_500 VSSC1thr ⌕

Gain	Threshold	Width
FSC	458 ▴ ▾	(1~3000)
VSSC1	100 ▴ ▾	(1~3000)
VSSC2	300 ▴ ▾	(1~3000)
BSSC	100 ▴ ▾	(1~3000)
YSSC	100 ▴ ▾	(1~3000)
RSSC	10 ▴ ▾	(1~3000)
V447	1000 ▴ ▾	(1~3000)
B531	600 ▴ ▾	(1~3000)
Y595	1500 ▴ ▾	(1~3000)
R670	1000 ▴ ▾	(1~3000)
R710	1 ▴ ▾	(1~3000)
R792	1 ▴ ▾	(1~3000)
Set As Default Default QC Gain		
Import from File... Import from Catalog...		
Export to File... Export to Catalog... Close		

Acq. Setting - rEV-GFP UN 1_500 ⌕

Gain	Threshold	Width
Primary Threshold (Trigger Level)		
Channel:	VSSC1 ▾	
Manual:	350 (>0) Height	

Acq. Setting - rEV-GFP+vFRed+81 -PB 1_500 Pethr600 ⌕

Gain	Threshold	Width
Primary Threshold (Trigger Level)		
Channel:	Y595 ▾	
Manual:	600 (>0) Height	

Acq. Setting - rEV-GFP+vFRed+81 -PB 1_500 B531thr... ⌕

Gain	Threshold	Width
Primary Threshold (Trigger Level)		
Channel:	B531 ▾	
Manual:	200 (>0) Height	

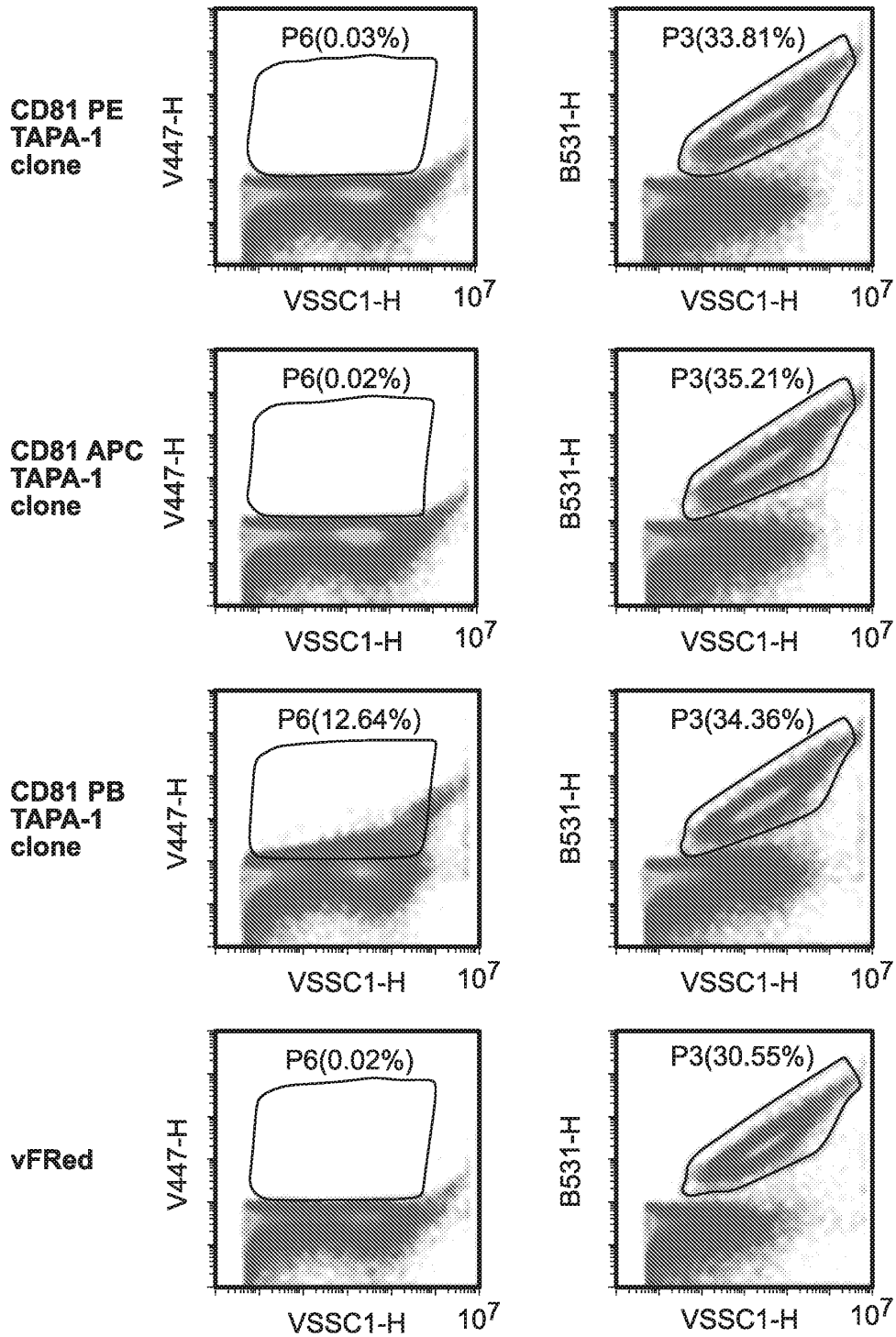
Acq. Setting - rEV-GFP+vFRed+81 -PB 1_500 V447thr... ⌕

Gain	Threshold	Width
Primary Threshold (Trigger Level)		
Channel:	V447 ▾	
Manual:	600 (>0) Height	

FIG. 47

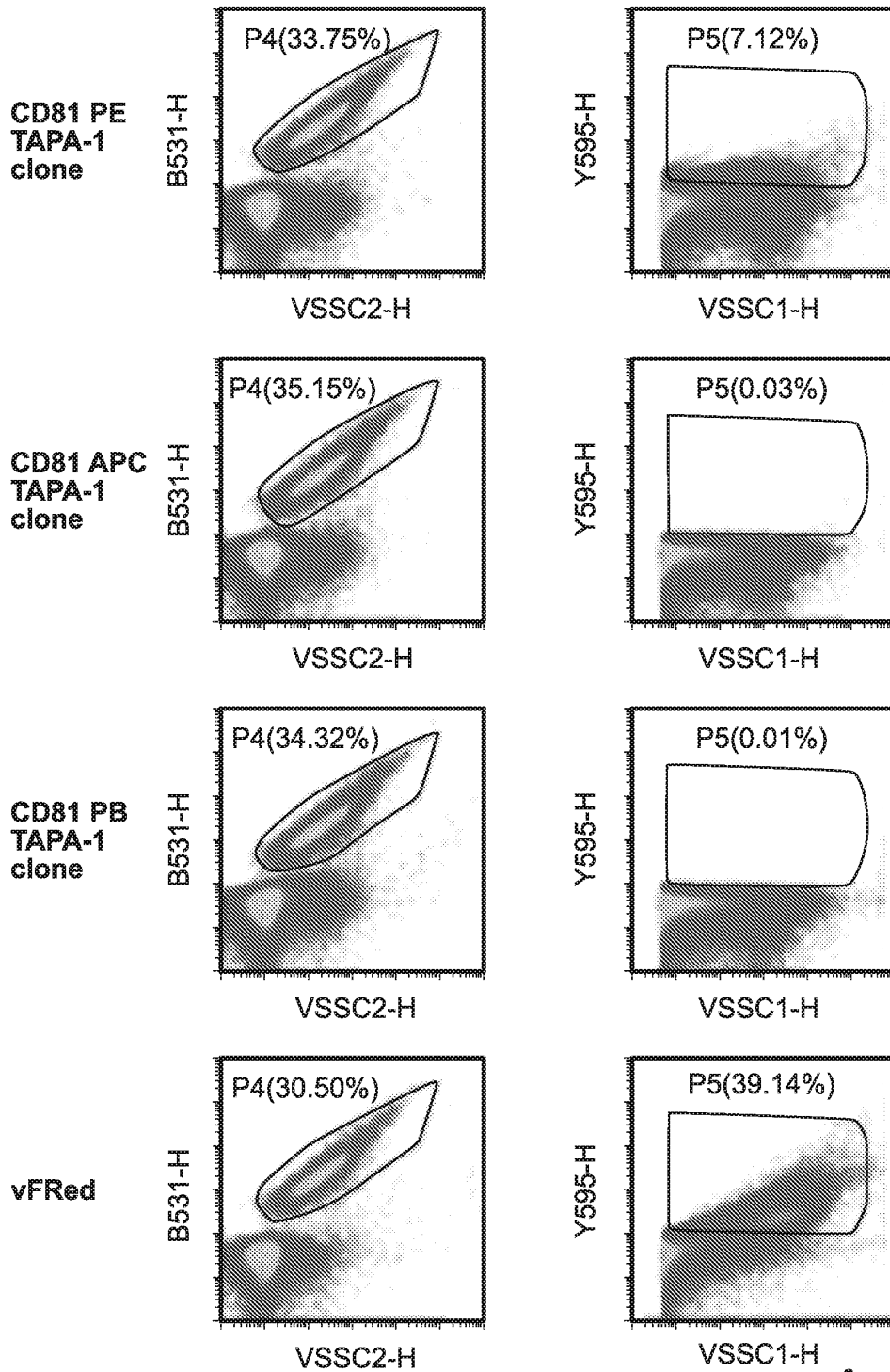
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VSSC1 trigger

**FIG. 48**

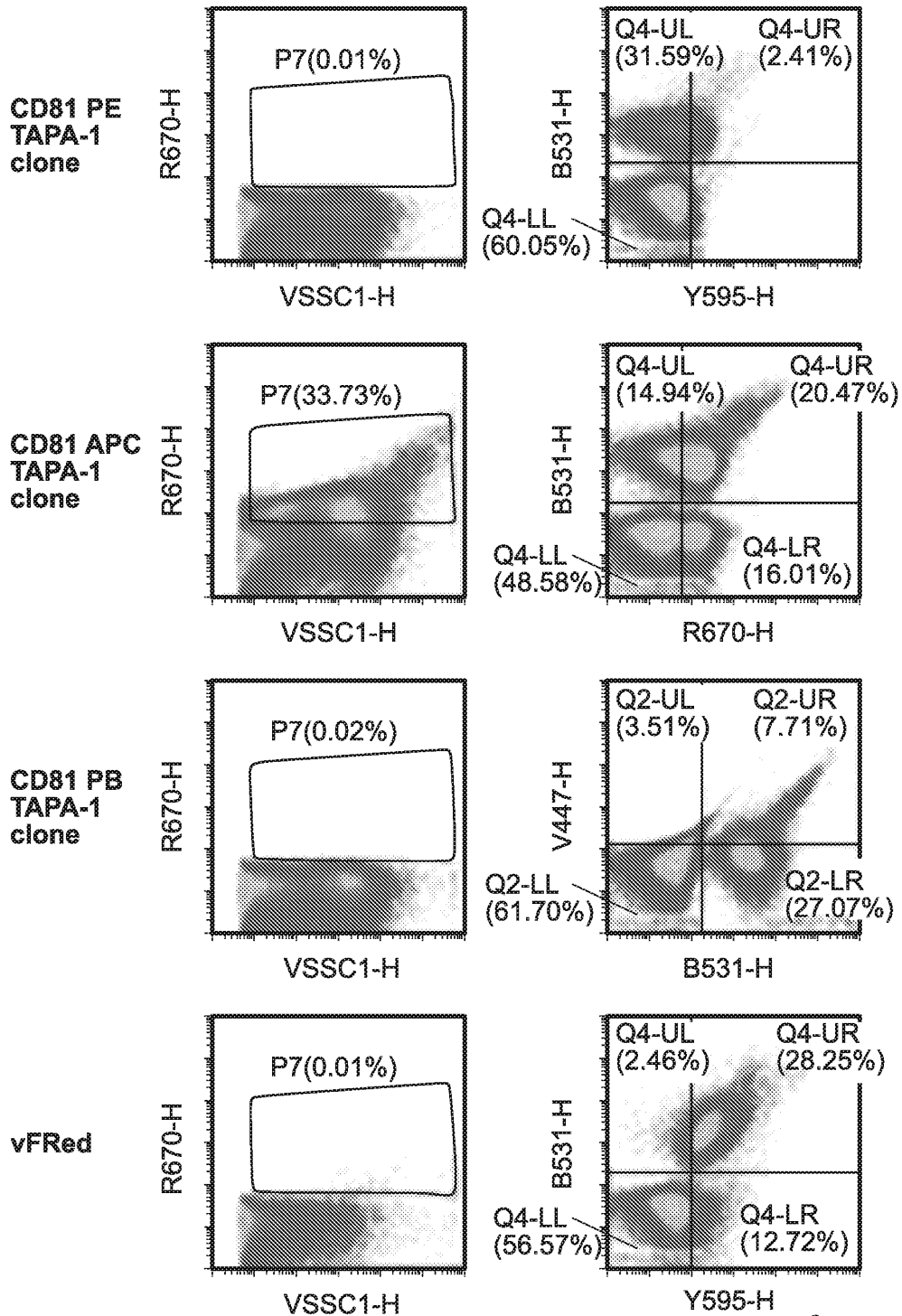
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VSSC1 trigger

**FIG. 48 (Cont. 1)**

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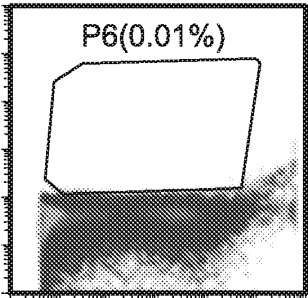
VSSC1 trigger

**FIG. 48 (Cont. 2)**

**VSSC1
trigger**

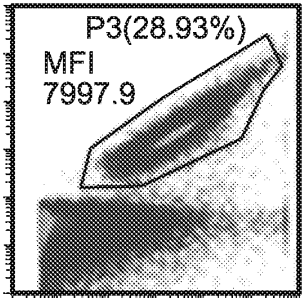
CD81 APC
TAPA-1 clone
+ vFRed

V447-H



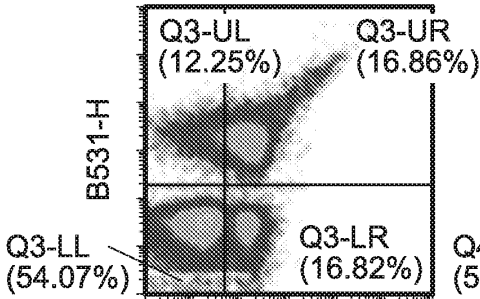
VSSC1-H

B531-H



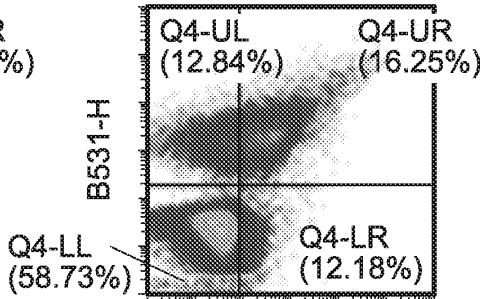
VSSC1-H

B531-H



R670-H

B531-H

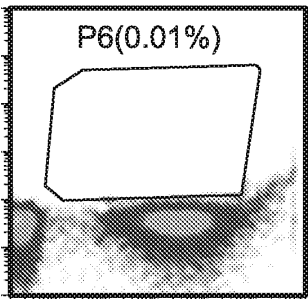


Y595-H

**B531
trigger**

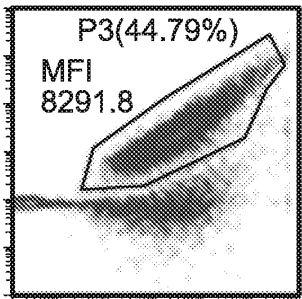
CD81 APC
TAPA-1 clone
+ vFRed

V447-H



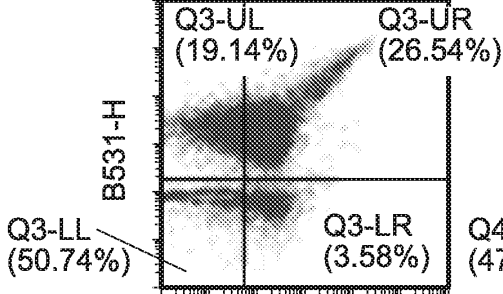
VSSC1-H

B531-H



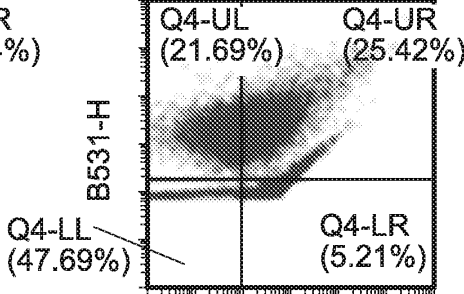
VSSC1-H

B531-H



R670-H

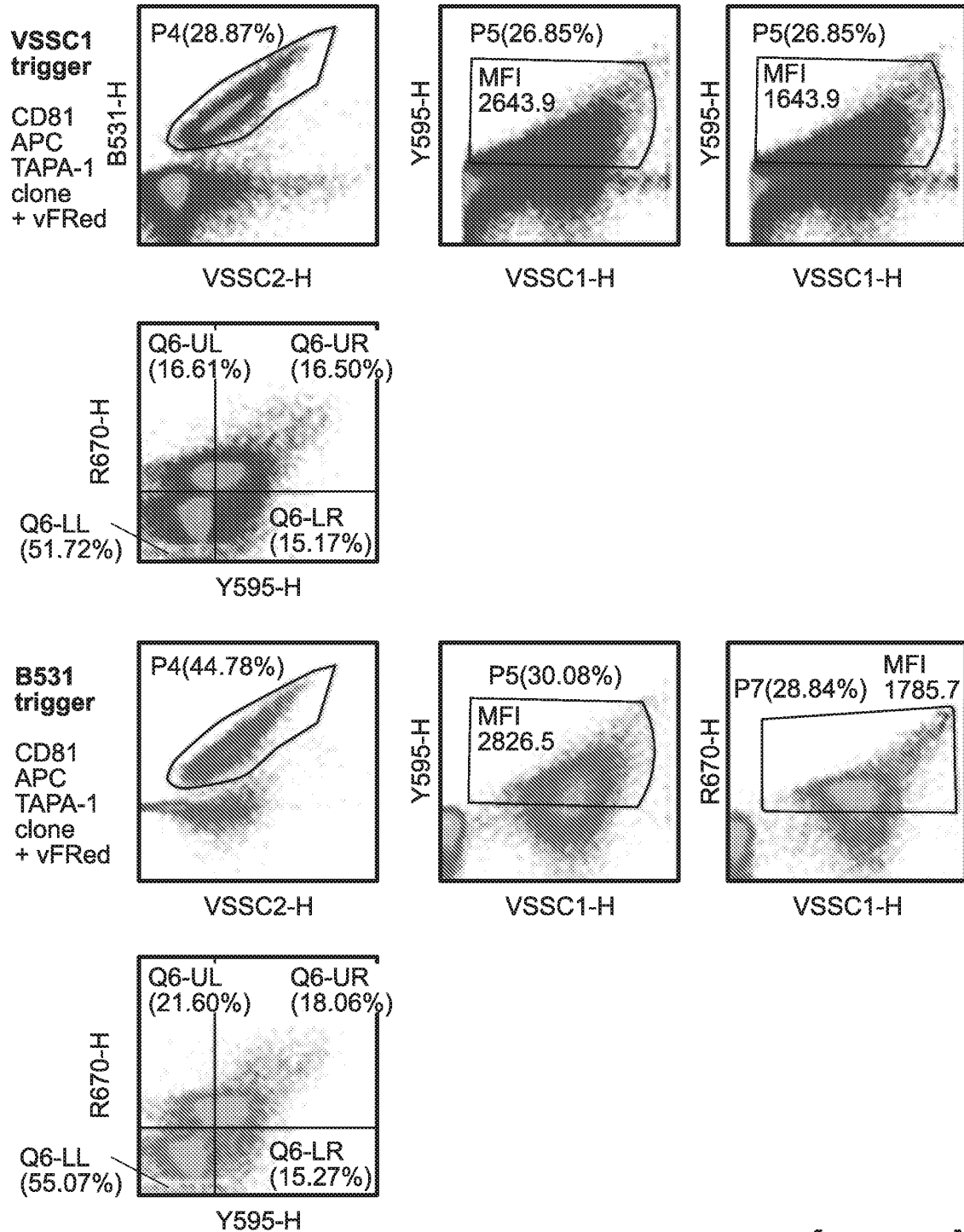
B531-H



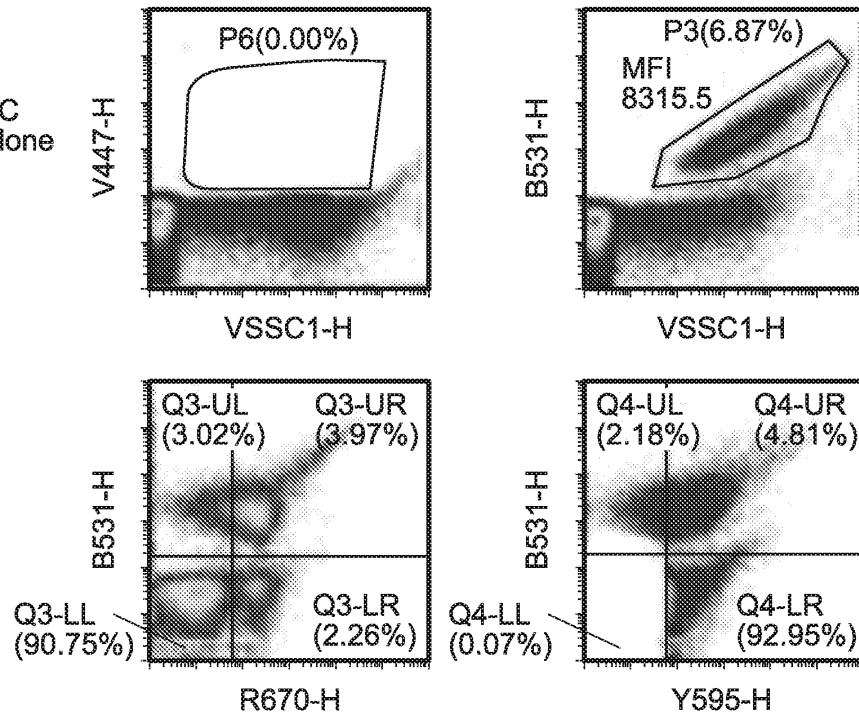
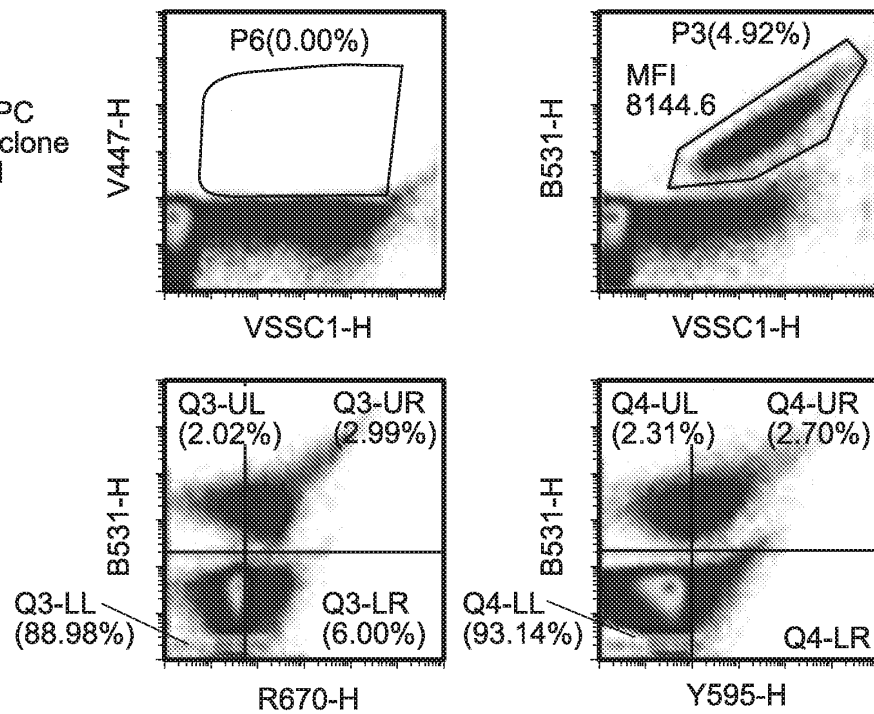
Y595-H

FIG. 49

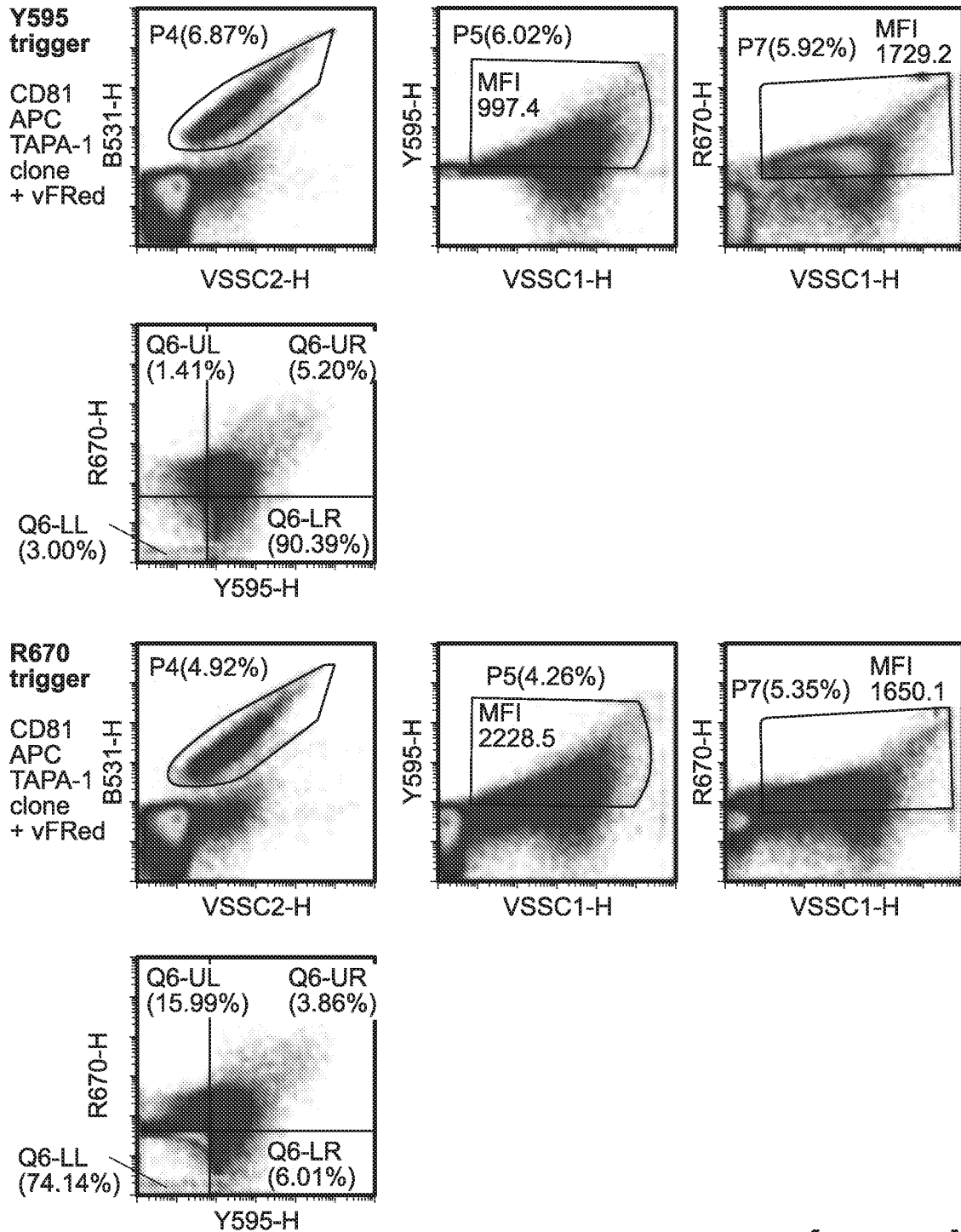
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**FIG. 49 (Cont.)**

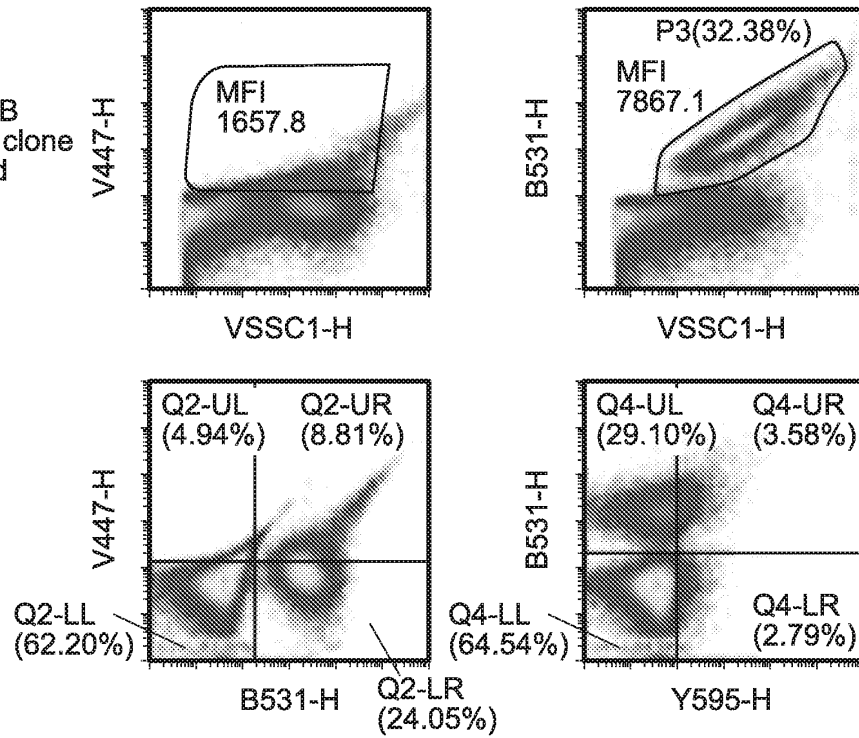
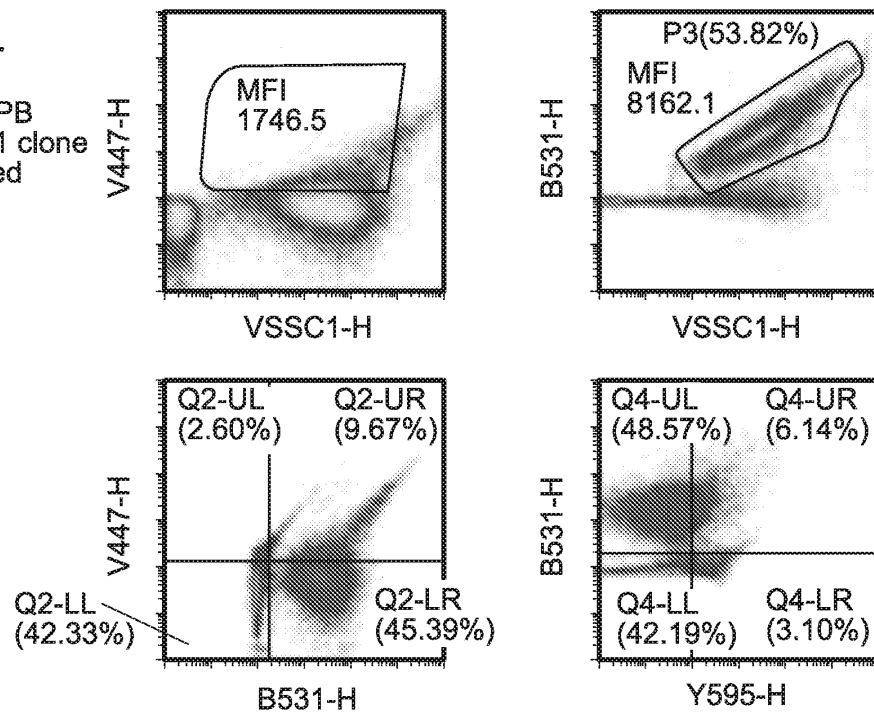
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**Y595
trigger**CD81 APC
TAPA-1 clone
+ vFRed**R670
trigger**CD81 APC
TAPA-1 clone
+ vFRed**FIG. 50**

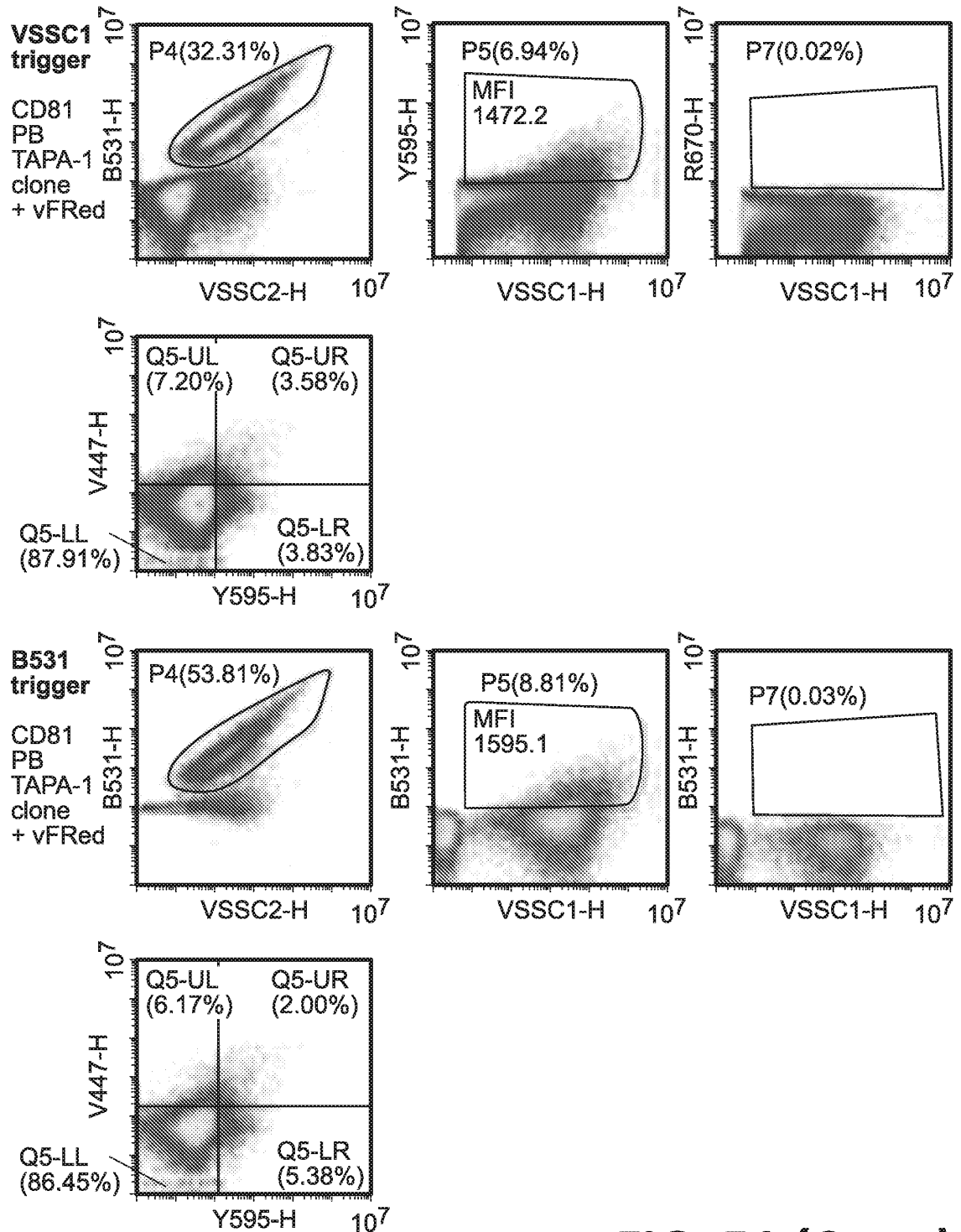
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**FIG. 50 (Cont.)**

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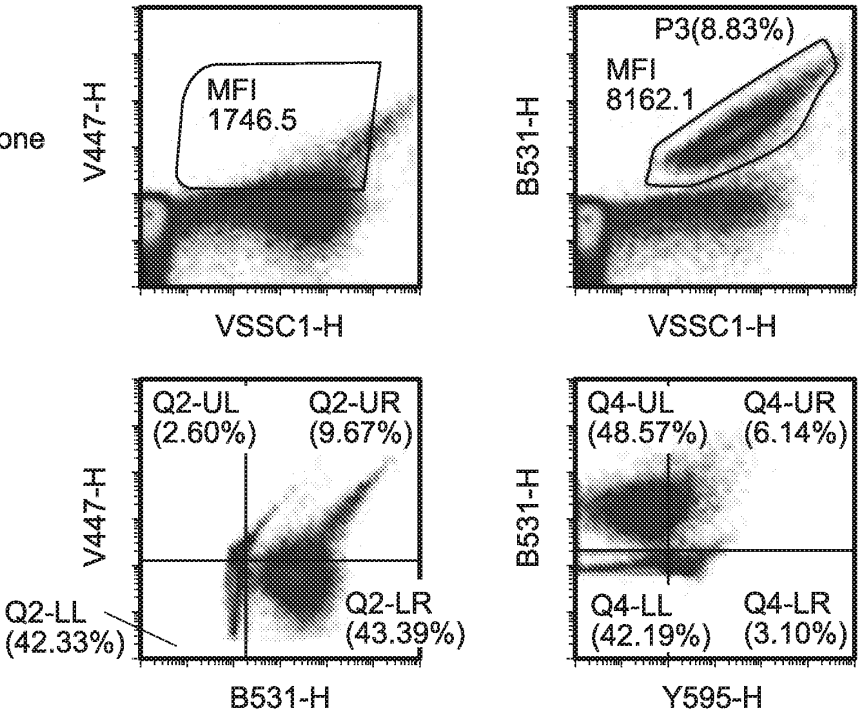
**VSSC1
trigger**CD81 PB
TAPA-1 clone
+ vFRed**B531
trigger**CD81 PB
TAPA-1 clone
+ vFRed**FIG. 51**

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**FIG. 51 (Cont.)**

**Y595
trigger**

CD81 PB
TAPA-1 clone
+ vFRed



**V447
trigger**

CD81 PB
TAPA-1 clone
+ vFRed

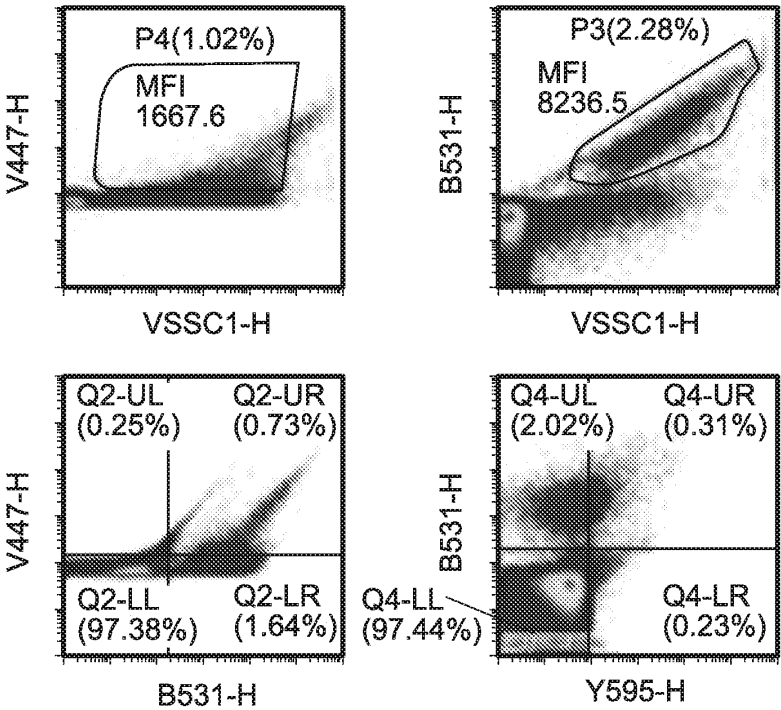
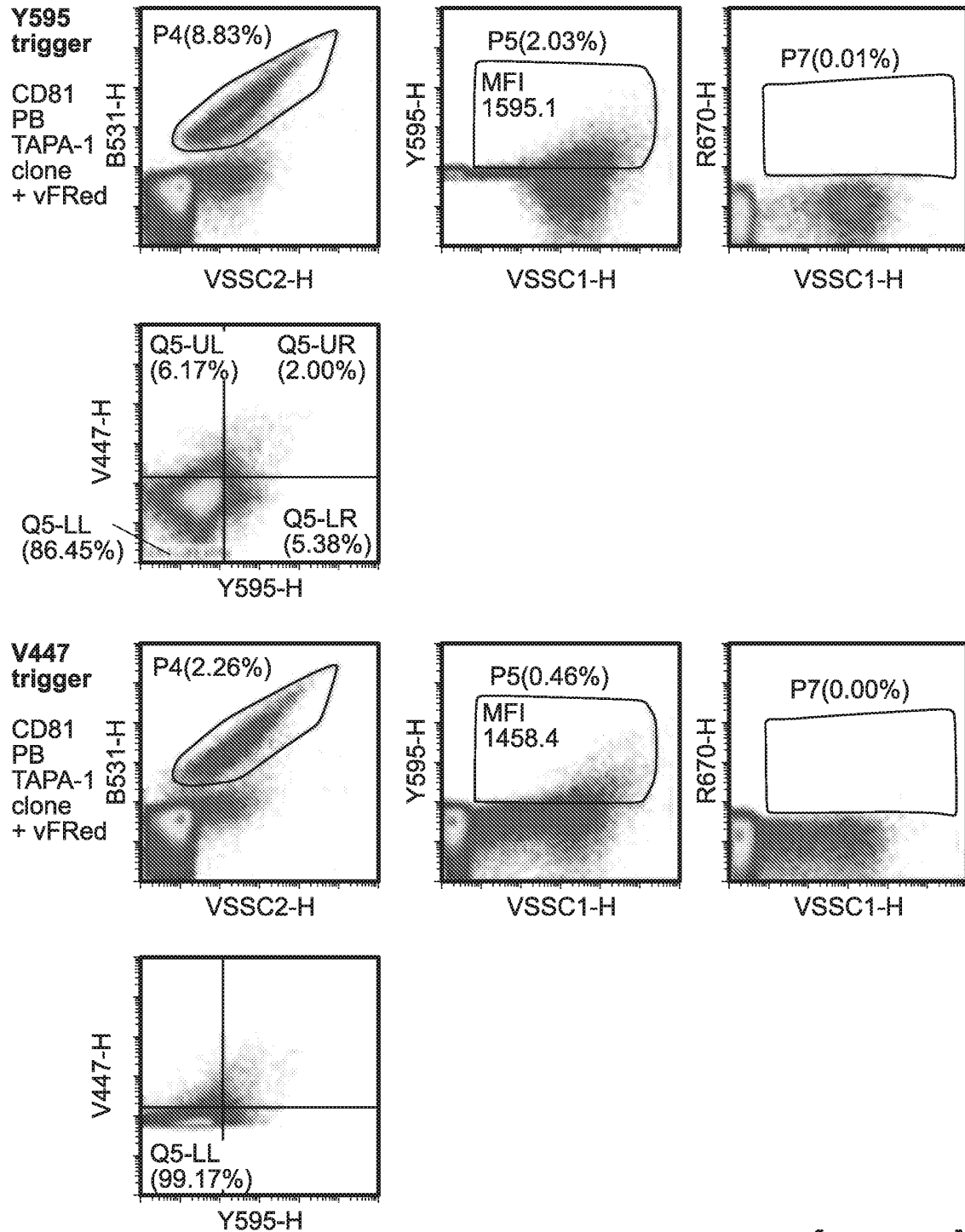
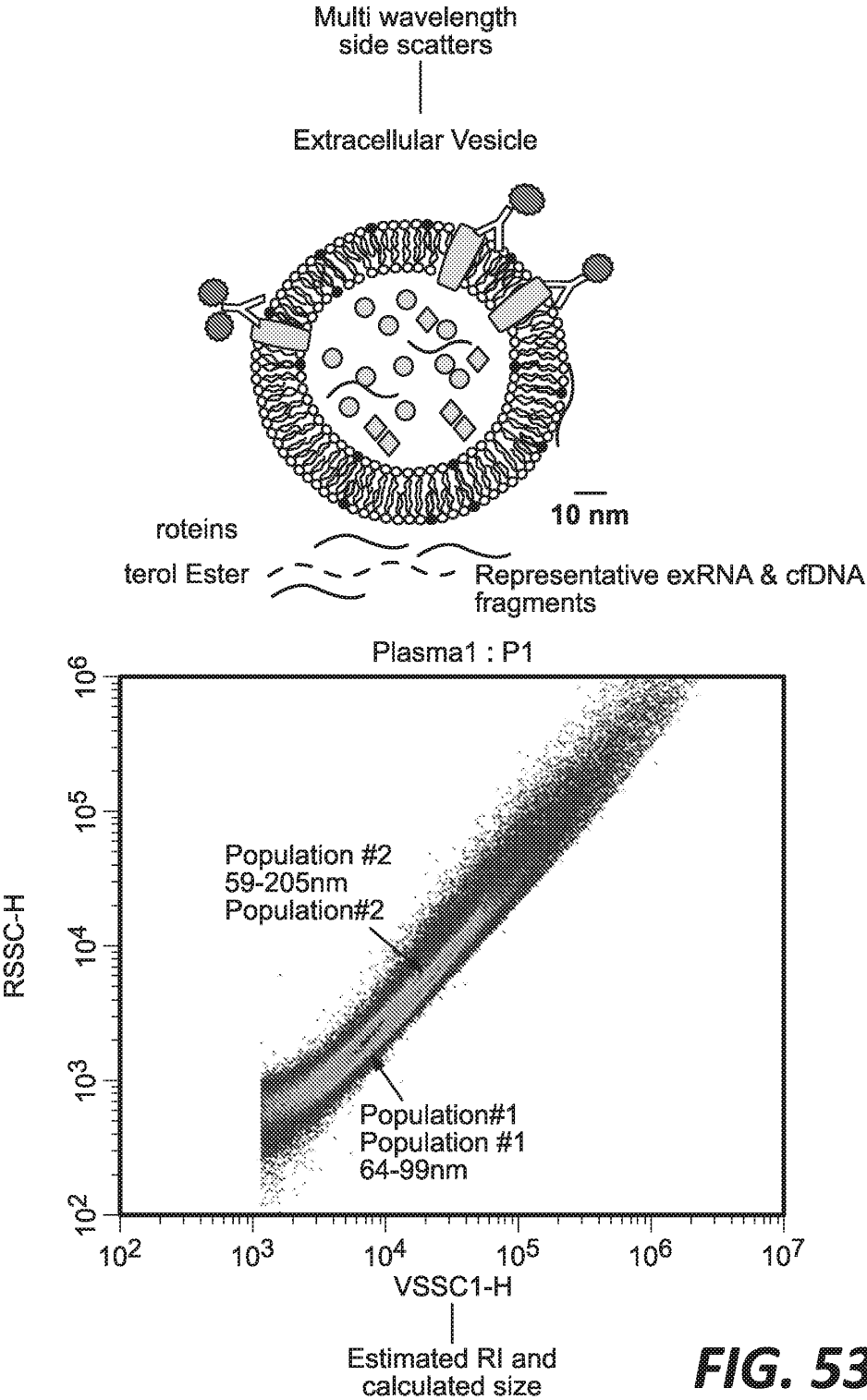


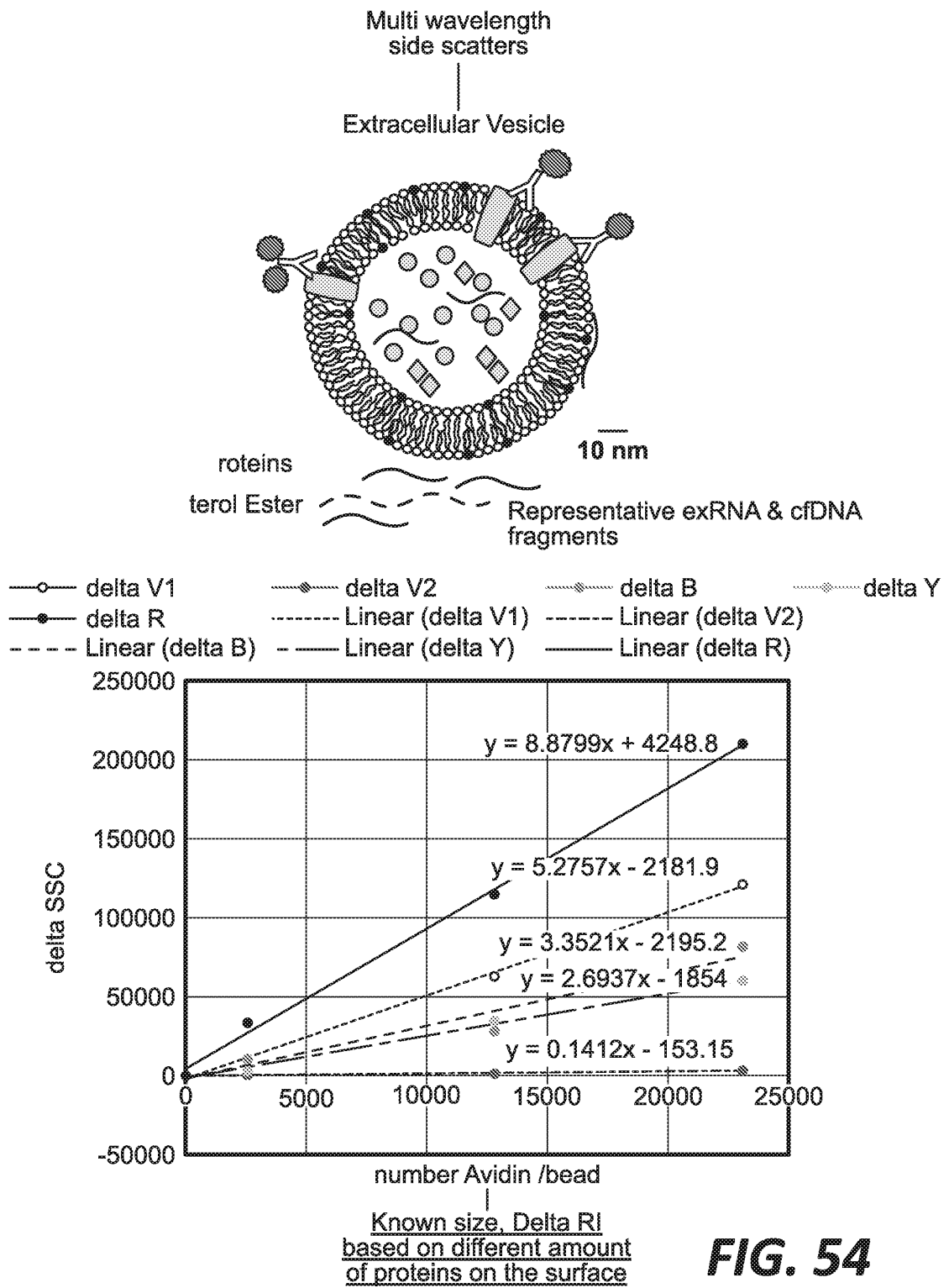
FIG. 52

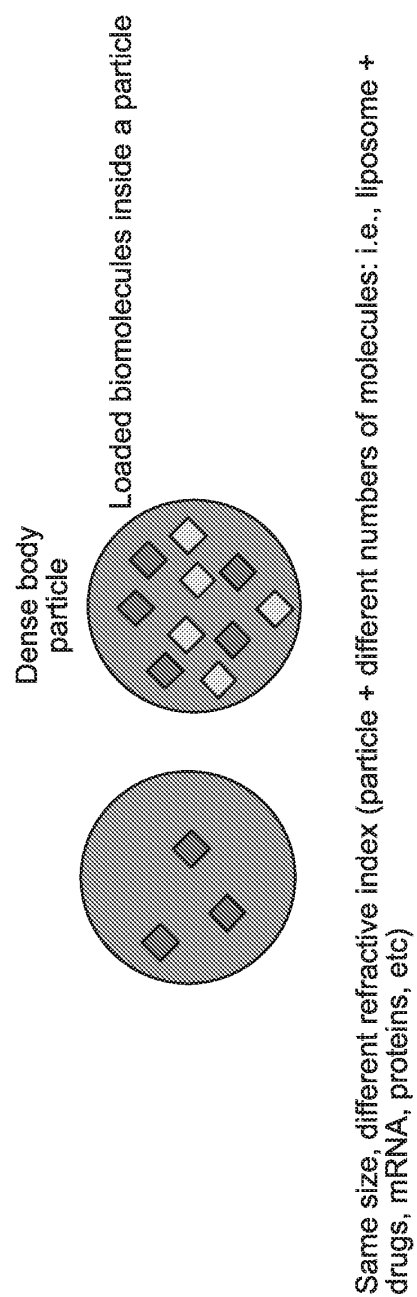
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**FIG. 52 (Cont.)**



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**FIG. 55**

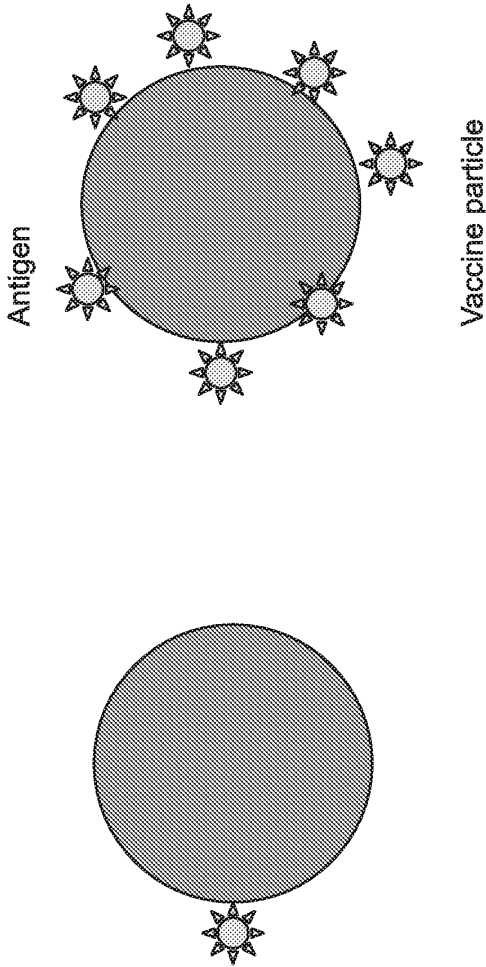


FIG. 56

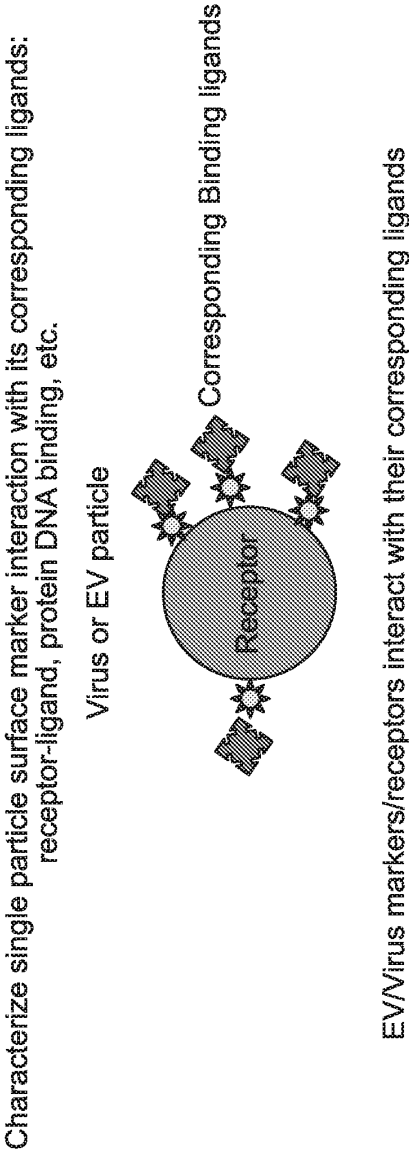


FIG. 57

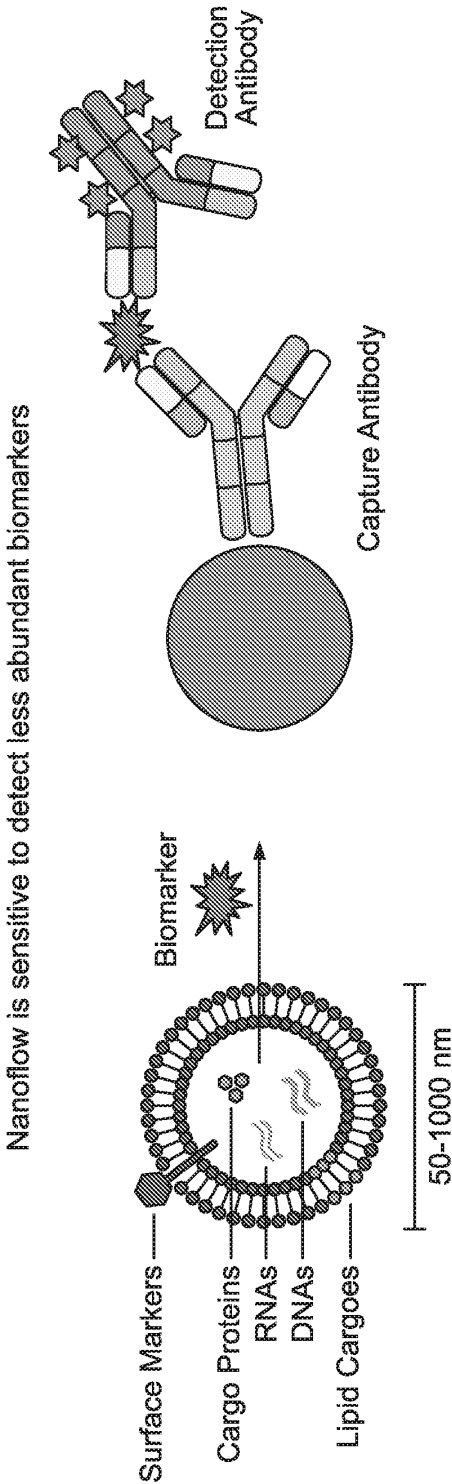
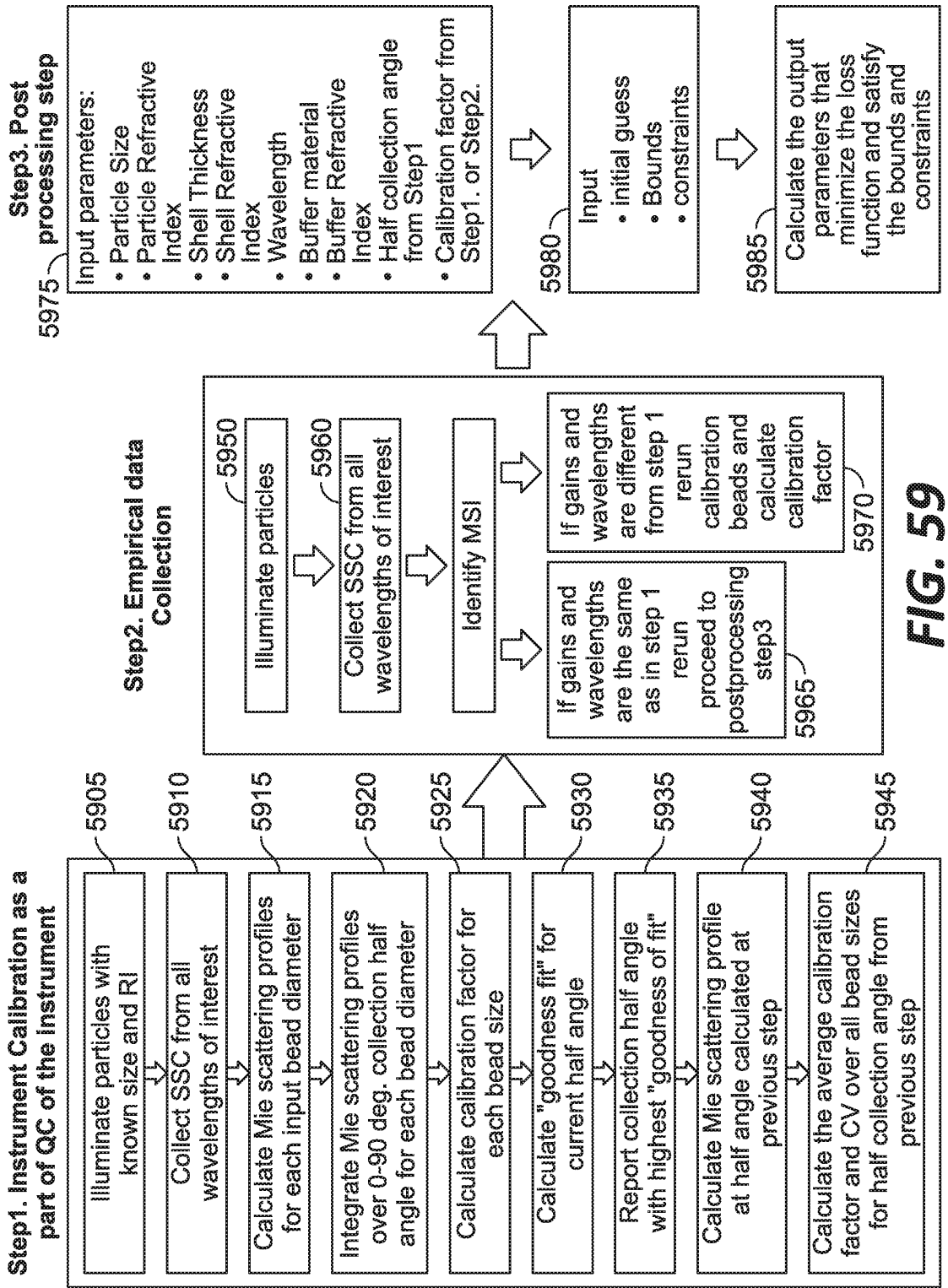


FIG. 58



Use cases				
Use Case	Particle type	Inputs	Outputs	
1 solid_ri	Solid	Particle diameter	Particle refractive index	
2 solid_size	Solid	Particle refractive index	Particle diameter	
3 coreshell_coresize	Core-shell	Shell thickness Shell refractive index Core refractive index	Core diameter	
4 coreshell_coresize	Core-shell	Core diameter Shell refractive index Core refractive index	Shell thickness	
5 coreshell_coreri	Core-shell	Shell refractive index Shell thickness Core diameter	Core refractive index	
6 coreshell_shellri	Core-shell	Core refractive index Shell thickness Core diameter	Shell refractive index	
7 coreshell_shellsize_coreri	Core-shell	Shell thickness Shell refractive index	Core diameter Core refractive index	
8 coreshell_shellsize_shellri	Core-shell	Core diameter Core refractive index	Shell thickness Shell refractive index	
9 coreshell_coresize_shellsize	Core-shell	Core refractive index Shell refractive index	Core diameter Shell thickness	
10 coreshell_shellsize_shellri	Core-shell	Core diameter Shell thickness	Core refractive index Shell refractive index	

FIG. 60

Algorithm parameters					
All algorithms were restricted to a maximum of 5,000 iterations					
Use Case	Minimization algorithm	Inputs	Outputs	Outputs	
solid_ri	Powell's algorithm	None	1.4	[1.33, 2.5]	
solid_size	Powell's algorithm	None	100 nm	[10 nm, 1500 nm]	
coreshell_coresize	Powell's algorithm	None	100 nm	[10 nm, 1500 nm]	
coreshell_shellsize	Powell's algorithm	None	10 nm	[0 nm, 1500 nm]	
coreshell_coreri	Powell's algorithm	None	1.4	[1.33, 2.5]	
coreshel_shellri	Powell's algorithm	None	1.4	[1.33, 2.5]	
coreshell_coresize_coreri	Powell's algorithm	None	Core RI: 1.4 Core diameter: 50 nm	Core RI: [1.33, 2.5] Core diameter: [10 nm, 1500 nm]	
coreshell_shellsize_shellri	Powell's algorithm	None	Shell RI: 1.4 Shell thickness: 10 nm	Shell RI: [1.33, 2.5] Shell thickness: [0 nm, 1500 nm]	
coreshell_coresize_shellsize	Constrained trust - region	shell thickness > core diameter	Core diameter: 50 nm Shell thickness: 75 nm	Core diameter: [10 nm, 1500 nm] Shell thickness: [0 nm, 1500 nm]	
	Constrained trust - region	shell thickness > core diameter	Core diameter: 100 nm Shell thickness: 10 nm		
	Powell's algorithm	None	Core diameter: 100 nm Shell thickness: 10 nm		
coreshel_coreri_shellri	Constrained trust - region	shell RI > core RI	Core RI: 1.4 Shell RI: 1.45	Core RI: [1.33, 2.5] Shell RI: [1.33, 2.5]	
	Constrained trust - region	shell RI > core RI	Core RI: 1.45 Shell RI: 1.4		
	Powell's algorithm	None	Core RI: 1.4 Shell RI: 1.45		

FIG. 61

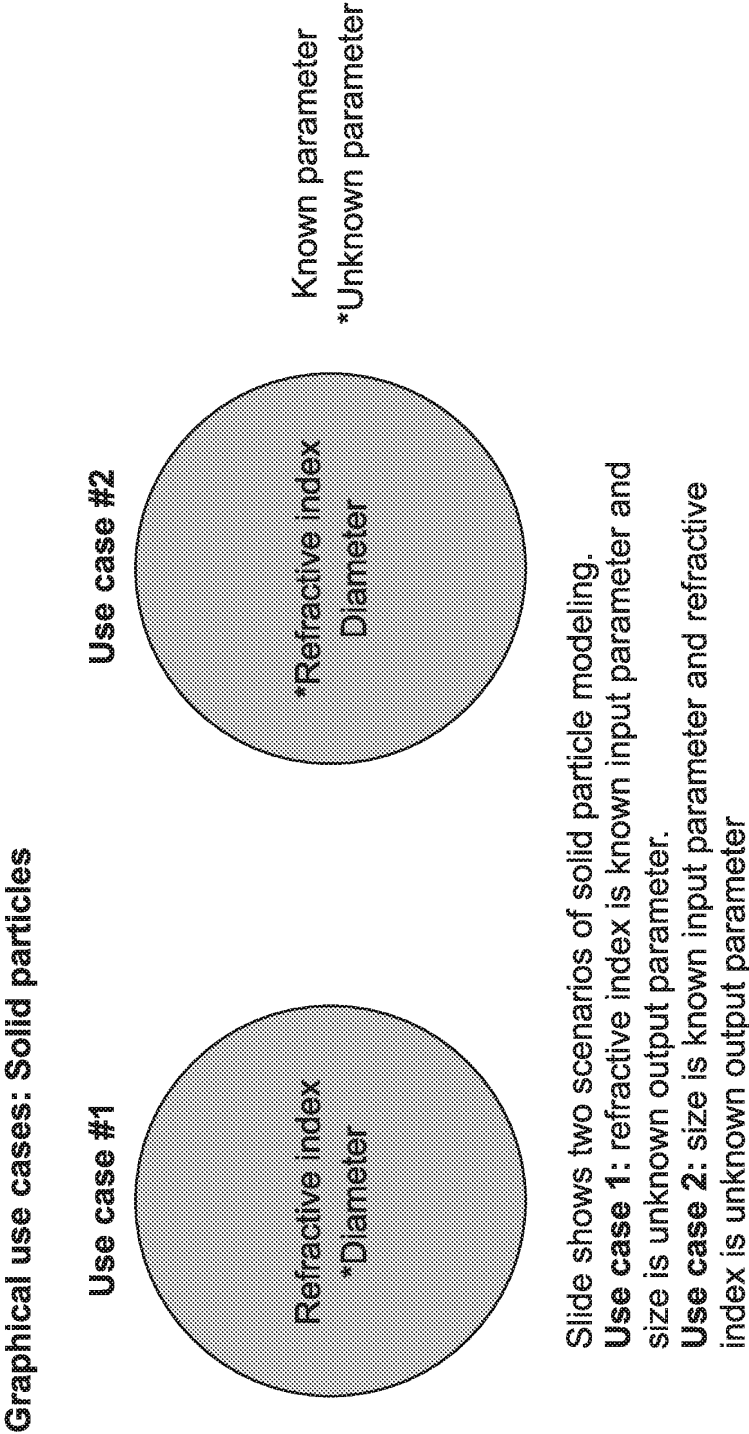
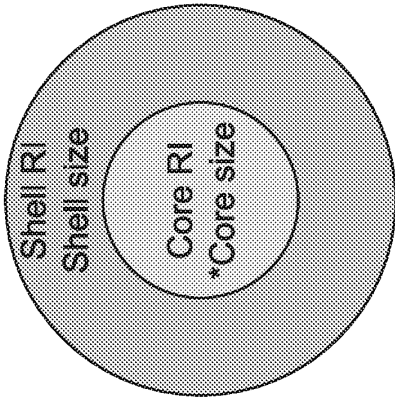


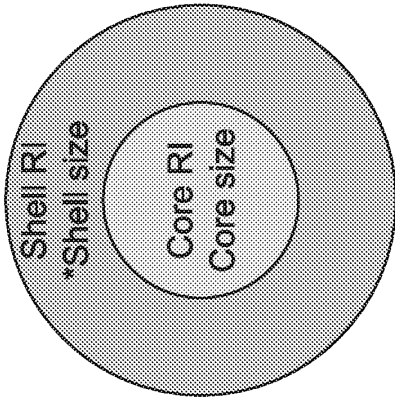
FIG. 62

Graphical use cases: core-shell particles with 1 unknown

Use case #3

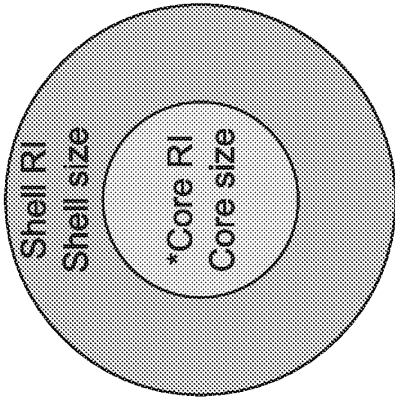


Use case #4



Known parameter
*Unknown parameter

Use case #5



Use case #6

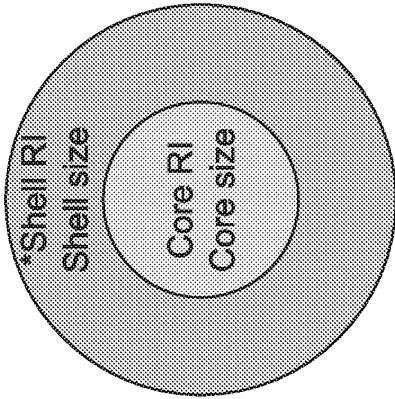


FIG. 63

Graphical use cases: core-shell particles with 2 unknowns

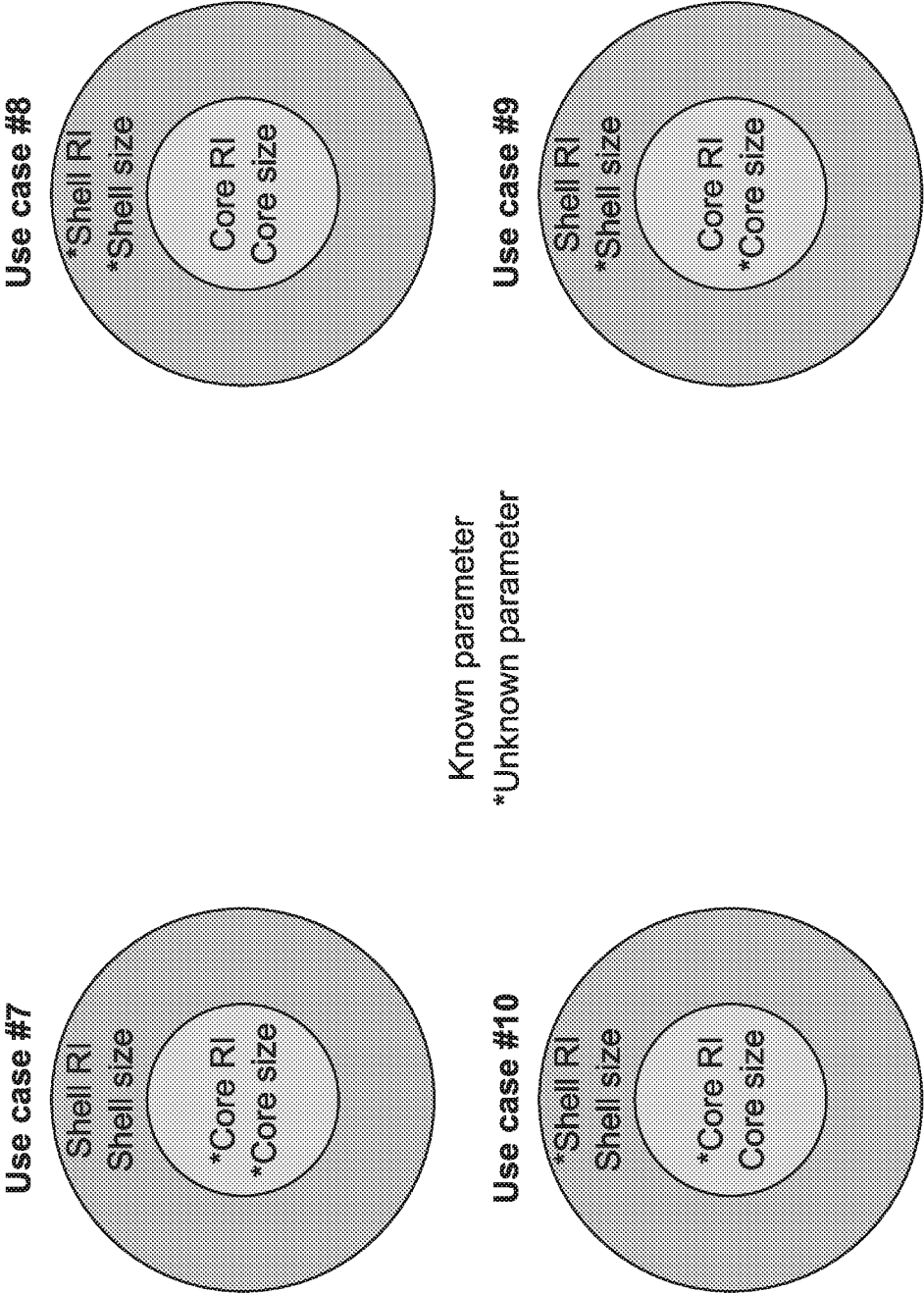
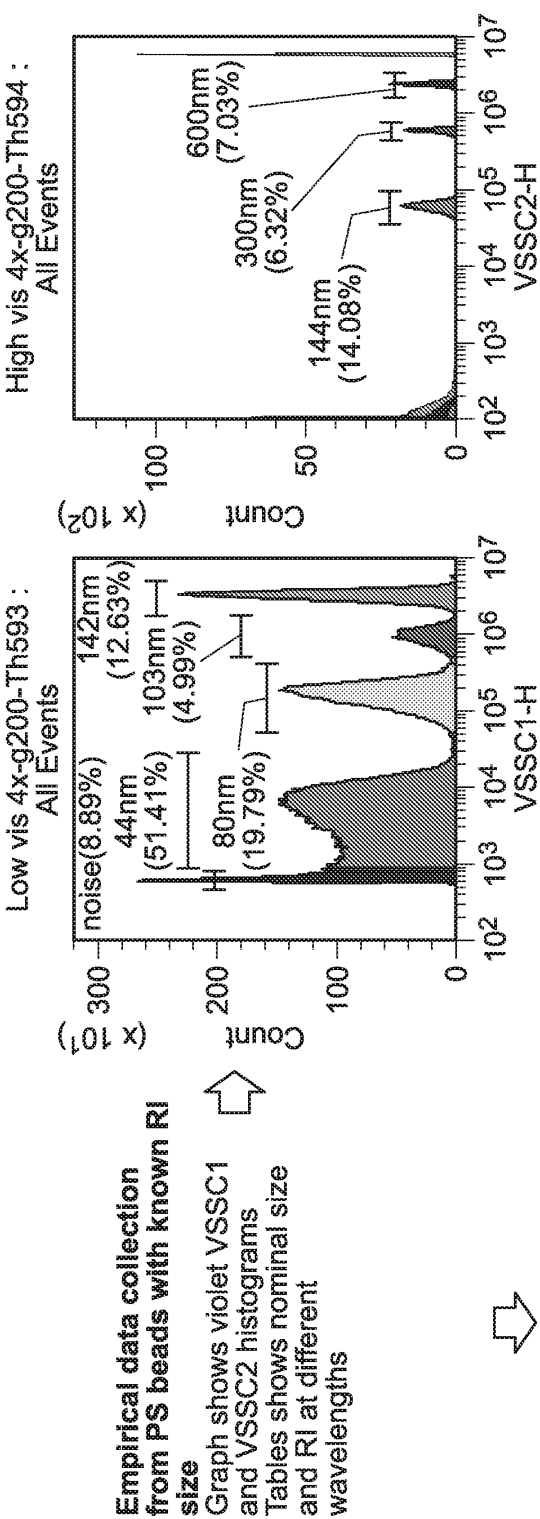


FIG. 64

Step 1. Instrument Calibration

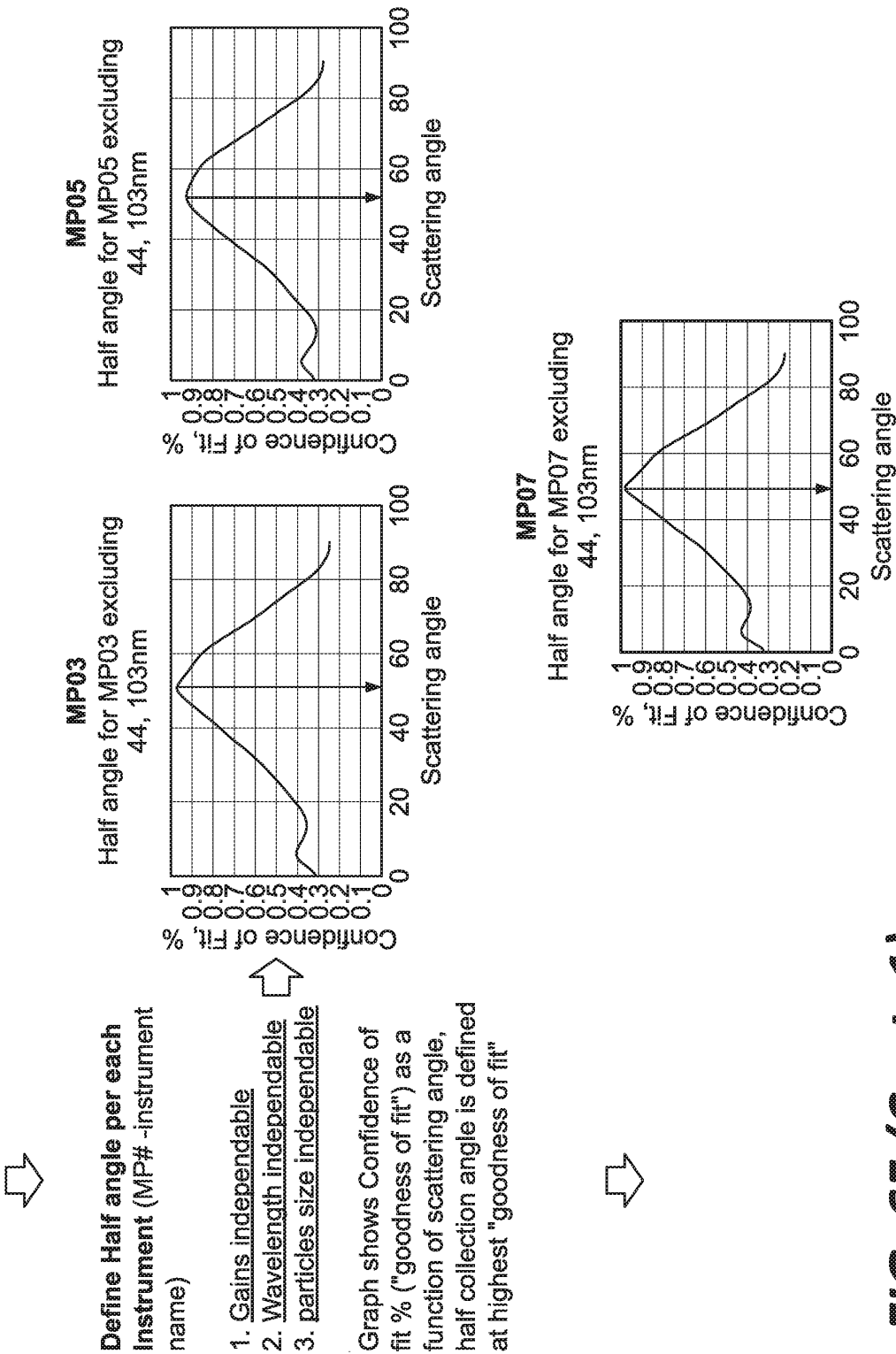


Polystyrene beads

Vendor	Nominal size, nm	CV, %
Low vis	40	11.5
Low vis	80	6.9
Low vis	103	4.8
Low vis	141	2.6
Hig vis	141	2.6
Hig vis	304	4.9
Hig vis	600	2.2
Hig vis	1020	2.6

Polystyrene beads	
wavelength	RI
405	1.6253
488	1.6039
561	1.5931
633	1.586

FIG. 65





Define calibration factor based on identified half angle

1. Gains dependable

2. Wavelength dependable

3. particle size in dependable



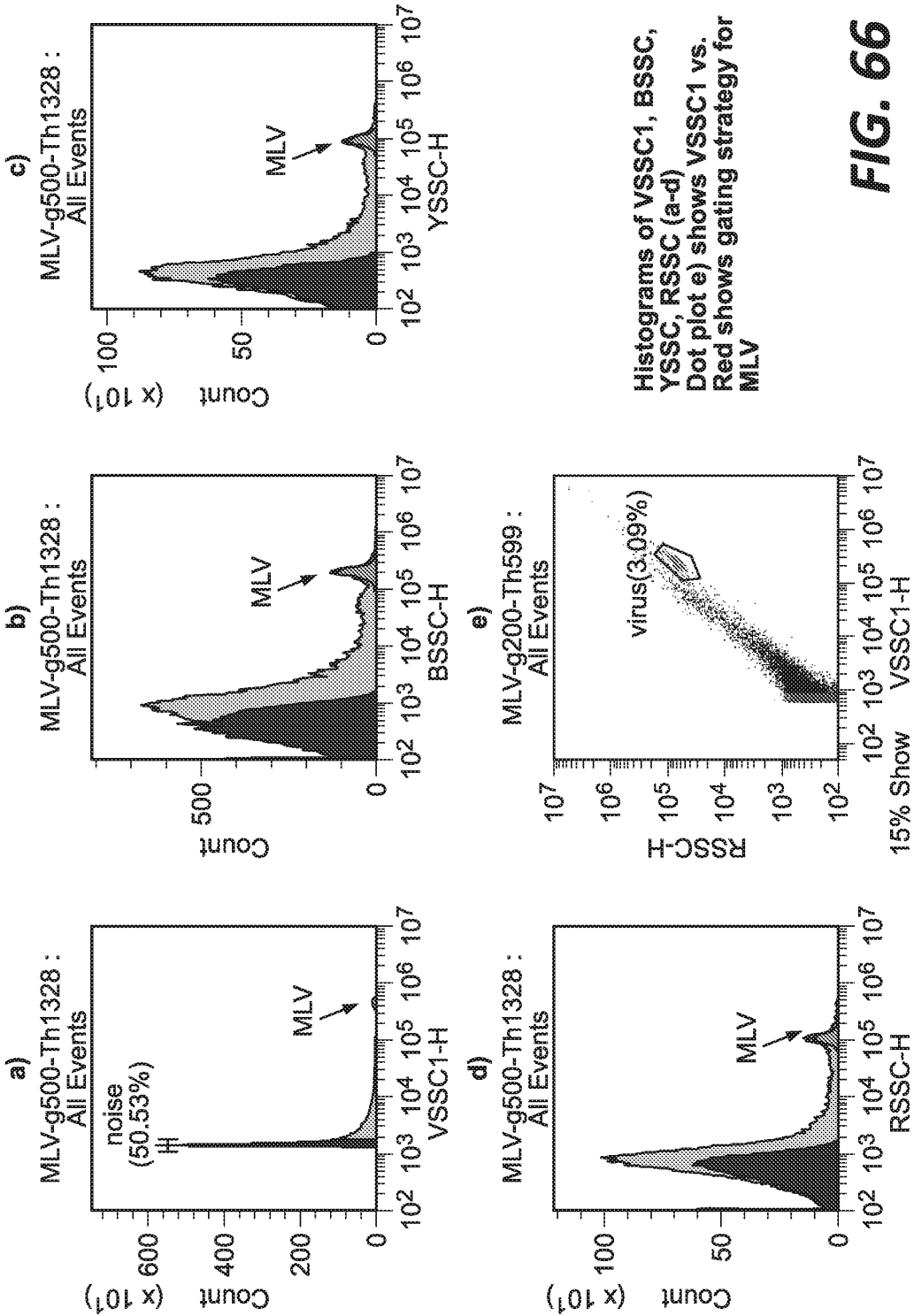
Table shows averaged calibration factors over all bead sizes at different wavelengths of scatter VSSC1 (violet), BSSC (blue), YSSC (yellow), RSSC (red)

Instrument	BSSC	RSSC	VSSC1	YSSC
MP05	7.45E+09	1.31E+10	9.6E+09	3.97E+09
MP07	5.84E+09	7.68E+09	7.23E+09	4.39E+09
MP03	5.56E+09	6.66E+09	3.79E+09	3.94E+09

FIG. 65 (Cont. 2)

SUBSTITUTE SHEET (RULE 26)

Step 2. Empirical data collection
Example of biological control #1: MLV virus commercially available



Step 2. Empirical data collection
Example of biological control #1: MLV virus commercially available

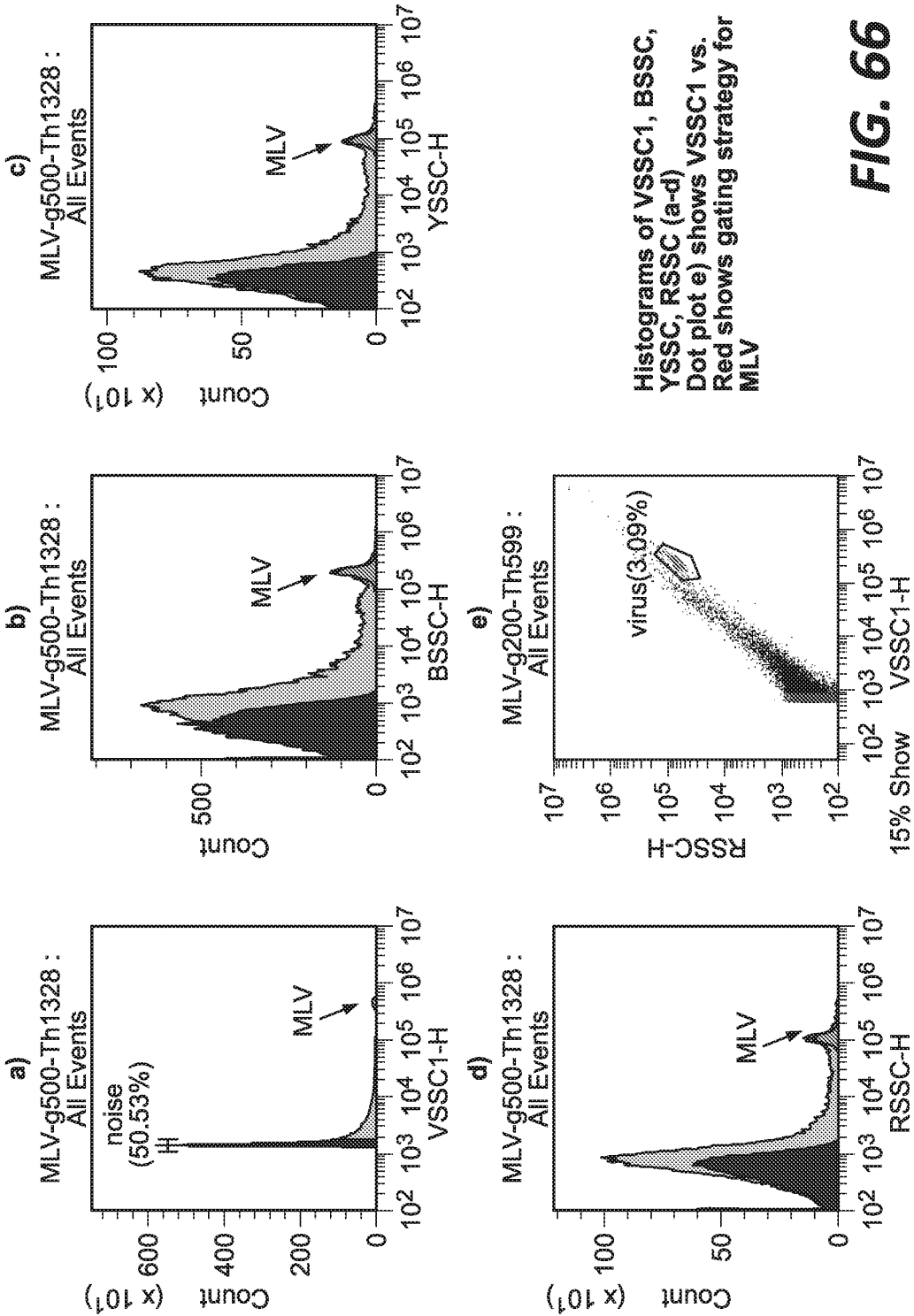


FIG. 66

Step 2. Empirical data collection
Example of biological control #2: recombinant EVs commercially available

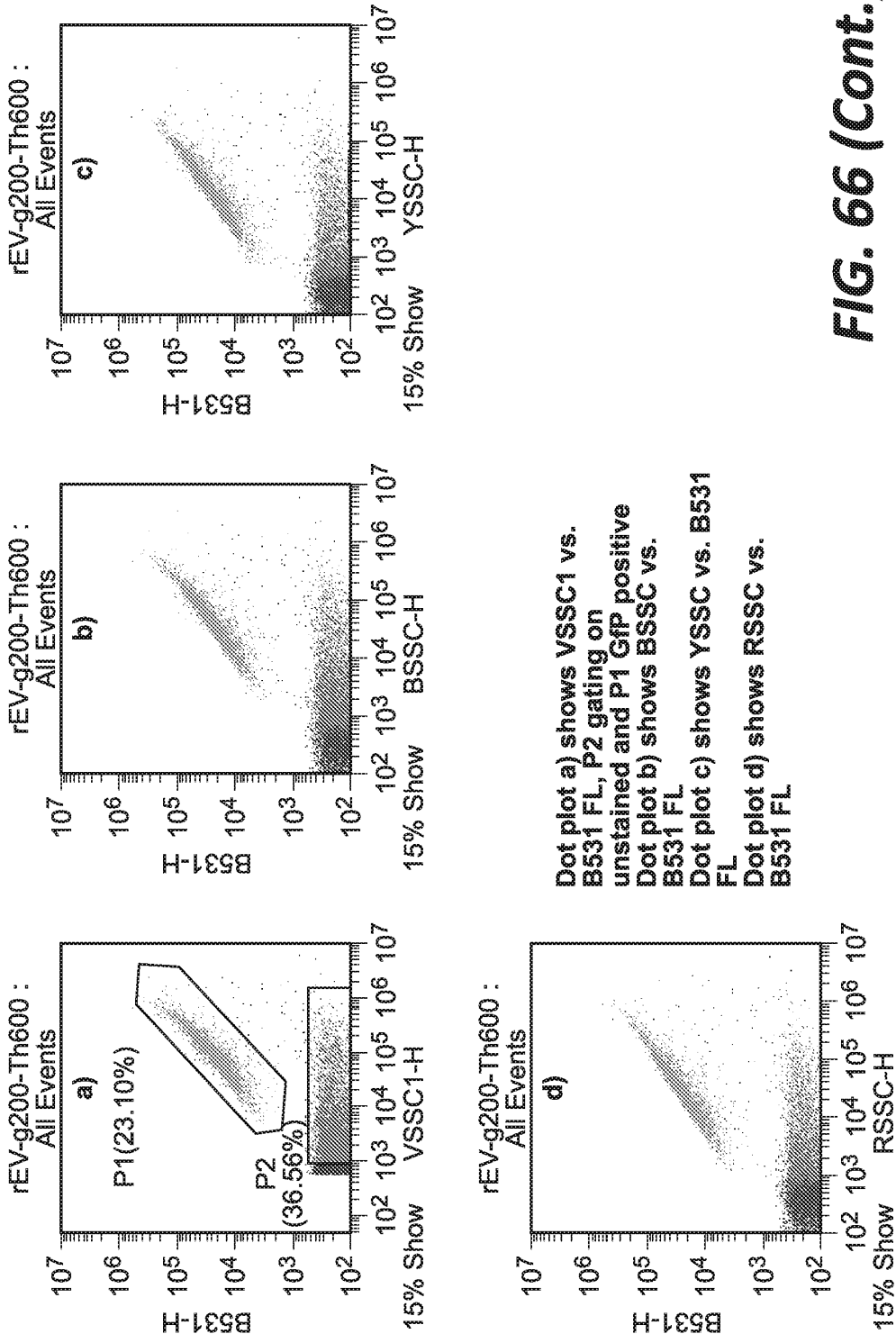


FIG. 66 (Cont.)

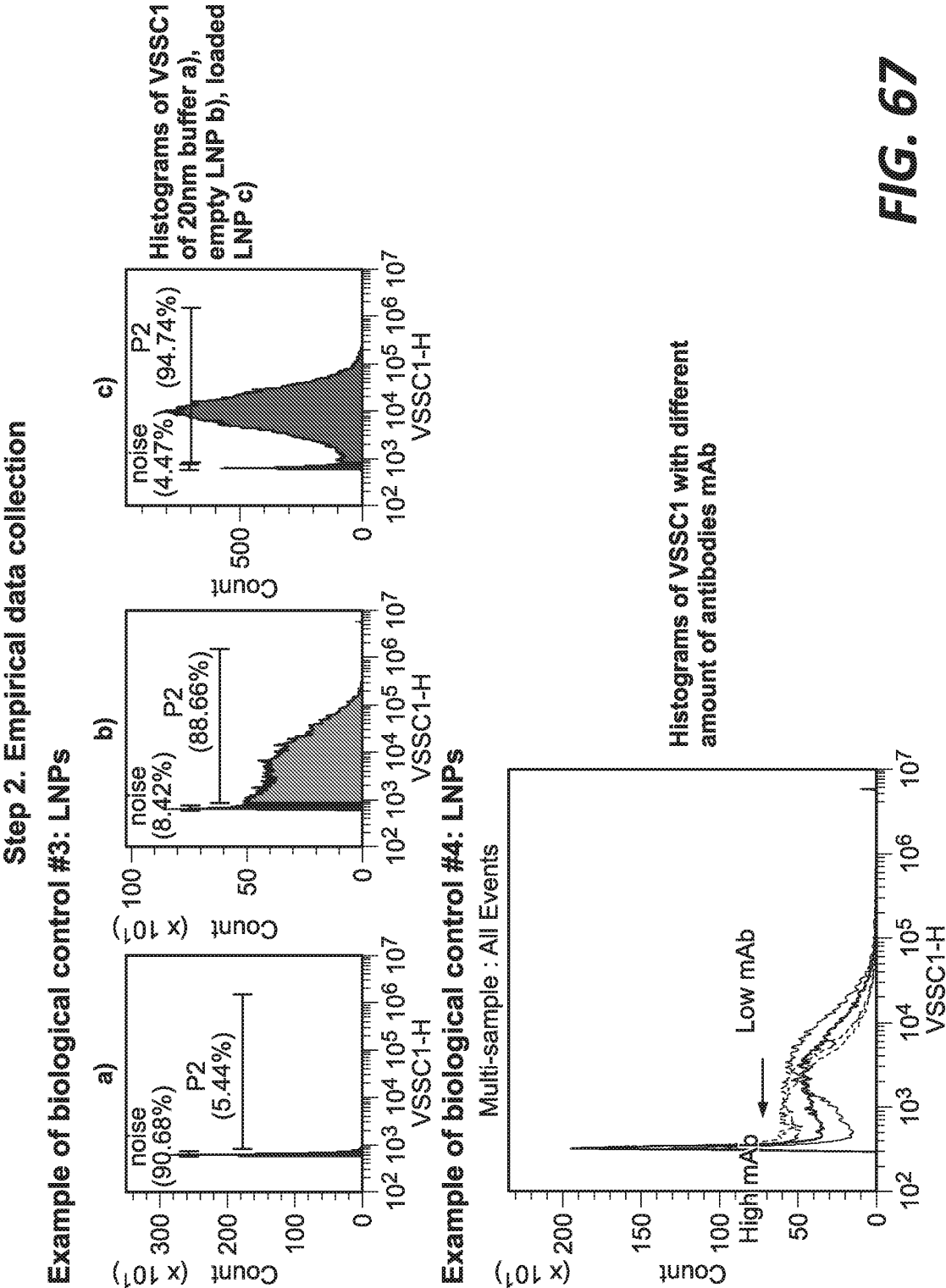


FIG. 67

Step 3. Postprocessing step

Input

Instrument	Particle type	Gain	Channel	Expected core diameter [nm]	Expected core RI	Empirical data
MP05	MLV	200	YSSC	124	1.435	35309.3
MP05	MLV	200	VSSC1	124	1.447	225423.2
MP05	MLV	200	RSSC	124	1.44	104156.5
MP05	MLV	200	BSSC	124	1.432	74312.4
MP07	MLV	200	YSSC	124	1.435	36554.2
MP07	MLV	200	VSSC1	124	1.447	169373.7
MP07	MLV	200	RSSC	124	1.44	78096.6
MP07	MLV	200	BSSC	124	1.432	42424.8
MP03	MLV	200	YSSC	124	1.435	33080.5
MP03	MLV	200	VSSC1	124	1.447	89585.2
MP03	MLV	200	RSSC	124	1.44	75509.3
MP03	MLV	200	BSSC	124	1.432	36784.7

Use case #1

- 3 instruments: MP05, MP07, MP03
- Particle type: MLV
- Gain 200 for all side scatter channels
- Channels: VSSC1 (violet), BSSC (blue), YSSC (yellow), RSSC (red)
- Expected size identified from Cryo TEM
- Input parameter: Refractive index
- Empirical data: medium scatter intensities from all side scatter channels
- Output parameter: Size
- predicted size error: comparison "Predicted - Expected":

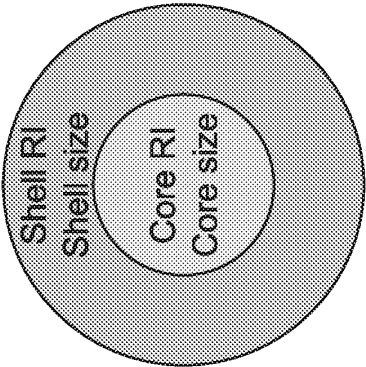


FIG. 68

Output	predicted size error
Predicted core RI	predicted core RI error
125.3118033	1.311803265
123.3249903	-0.675009731
124.0085441	0.008544083
125.3708504	1.370850431
124.7097627	0.709762721
124.3565028	0.356502833
123.8995593	-0.100440698
125.6166387	1.616638669
124.4277141	0.427714098
124.0595608	0.059560813
123.822878	-0.17712197
125.1883364	1.188336395

FIG. 68 (Cont.)

Step 3. Postprocessing step

Input

Instrument	Particle type	Gain	Channel	Expected core diameter [nm]	Expected core RI	Empirical data
MP05	MLV	200	YSSC	124	1.435	35309.3
MP05	MLV	200	VSSC1	124	1.447	225423.2
MP05	MLV	200	RSSC	124	1.44	104156.5
MP05	MLV	200	BSSC	124	1.432	74312.4
MP07	MLV	200	YSSC	124	1.435	36554.2
MP07	MLV	200	VSSC1	124	1.447	169373.7
MP07	MLV	200	RSSC	124	1.44	78096.6
MP07	MLV	200	BSSC	124	1.432	42424.8
MP03	MLV	200	YSSC	124	1.435	33080.5
MP03	MLV	200	VSSC1	124	1.447	89585.2
MP03	MLV	200	RSSC	124	1.44	75509.3
MP03	MLV	200	BSSC	124	1.432	36784.7

Use case #2

- 3 instruments: MP05, MP07, MP03
- Particle type: MLV
- Gain 200 for all side scatter channels
- Channels: VSSC1 (violet), BSSC (blue), YSSC (yellow), RSSC (red)
- Expected RI
- Input parameter: size
- Empirical data: medium scatter intensities from all side scatter channels
- Output parameter: RI
- predicted RI error: comparison "Predicted - Expected":

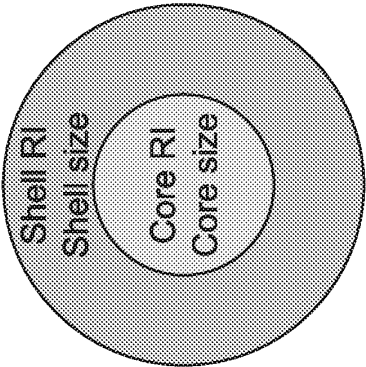


FIG. 69

Output	predicted RI error
Predicted core RI	Predicted core RI error
1.437927873	0.002927873
1.44570176	-0.00129824
1.440041461	4.15E-05
1.43510254	0.00310254
1.436563663	0.001563663
1.447694803	0.000694803
1.439784538	-0.000215462
1.435630154	0.003630154
1.435968244	0.000968244
1.447135132	0.000135132
1.439644359	-0.000355641
1.43469105	0.00269105

FIG. 69 (Cont.)

Step 3. Postprocessing step

Input				Input		
Instrument	Particle type	Gain	Channel	Expected core diameter [nm]	Expected shell thickness [nm]	Expected core RI / Expected shell RI
MP05	rEV	200	YSSC	98	5	1.4149 / 1.4863
MP05	rEV	200	VSSC1	98	5	1.4274 / 1.4863
MP05	rEV	200	RSSC	98	5	1.4121 / 1.4863
MP05	rEV	200	BSSC	98	5	1.4189 / 1.4863
MP07	rEV	200	YSSC	98	5	1.4149 / 1.4863
MP07	rEV	200	VSSC1	98	5	1.4274 / 1.4863
MP07	rEV	200	RSSC	98	5	1.4121 / 1.4863
MP07	rEV	200	BSSC	98	5	1.4189 / 1.4863
MP03	rEV	200	YSSC	98	5	1.4149 / 1.4863
MP03	rEV	200	VSSC1	98	5	1.4274 / 1.4863
MP03	rEV	200	RSSC	98	5	1.4121 / 1.4863
MP03	rEV	200	BSSC	98	5	1.4189 / 1.4863

Use case #5

- 3 instruments: MP05, MP07, MP03
- Particle type: rEV
- Gain 200 for all side scatter channels
- Channels: VSSC1 (violet), BSSC (blue), YSSC (yellow), RSSC (red)
- Expected: Core RI
- Input parameter: Core size, shell thickness, shell RI
- Empirical data: medium scatter intensities from all side scatter channels (MSI)
- Output parameter: Core RI
- predicted Core RI error: comparison "Predicted Core RI - Expected Core RI"

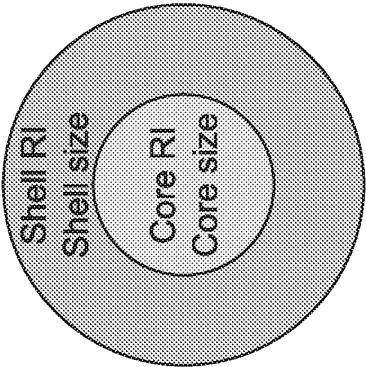


FIG. 70

Output		
Empirical data	Predicted core RI	Predicted core RI error
13175.3	1.408560962	-0.006339038
87254.5	1.418265215	-0.009134785
26857.4	1.403703219	-0.008396781
39461.2	1.411356644	-0.007543356
15519.4	1.414931375	3.14E-05
73821.6	1.427443273	4.33E-05
17399.1	1.412144978	4.50E-05
33676.9	1.418950678	5.07E-05
12638.1	1.407680356	-0.007219644
34894.4	1.420190969	-0.007209031
13445.1	1.403893447	-0.008206553
28907.4	1.411503676	-0.007396324

FIG. 70 (Cont.)

Step 3. Postprocessing step
Input

Instrument	Particle type	Gain	Channel	Expected core diameter [nm]	Expected shell thickness [nm]	Expected core RI	Expected shell RI
MP05	rEV	200	YSSC	98	5	1.4149	1.4863
MP05	rEV	200	VSSC1	98	5	1.4274	1.4863
MP05	rEV	200	RSSC	98	5	1.4121	1.4863
MP05	rEV	200	BSSC	98	5	1.4189	1.4863
MP07	rEV	200	YSSC	98	5	1.4149	1.4863
MP07	rEV	200	VSSC1	98	5	1.4274	1.4863
MP07	rEV	200	RSSC	98	5	1.4121	1.4863
MP07	rEV	200	BSSC	98	5	1.4189	1.4863
MP03	rEV	200	YSSC	98	5	1.4149	1.4863
MP03	rEV	200	VSSC1	98	5	1.4274	1.4863
MP03	rEV	200	RSSC	98	5	1.4121	1.4863
MP03	rEV	200	BSSC	98	5	1.4189	1.4863

Use case #10

- 3 instruments: MP05, MP07, MP03
- Particle type: rEV
- Gain 200 for all side scatter channels
- Channels: VSSC1 (violet), BSSC (blue), YSSC (yellow), RSSC (red)
- Expected: Core RI, Shell RI
- Input parameter: Core size, Shell thickness
- Empirical data: medium scatter intensities from all side scatter channels (MSI)
- Output parameter: Core RI, Shell RI
- predicted Core RI error: comparison "Predicted Core RI - Expected Core RI"
- predicted Shell RI error: comparison "Predicted Shell RI - Expected Shell RI"
- Constraints were used: Shell RI > Core RI

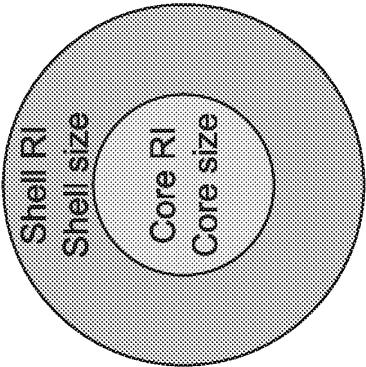


FIG. 71

Empirical data	Constraint	Output		Output	
		Predicted core RI	Predicted core RI error	Predicted shell RI	Predicted core RI error
13175.3	SHELL_RI_BIGGER_THAN_CORE_RI	1.409702495	-0.005197505	1.482375871	-0.003924129
87254.5	SHELL_RI_BIGGER_THAN_CORE_RI	1.416164806	-0.011235194	1.49403312	0.00773312
26857.4	SHELL_RI_BIGGER_THAN_CORE_RI	1.406318297	-0.005781703	1.477656887	-0.008643113
39461.2	SHELL_RI_BIGGER_THAN_CORE_RI	1.411650365	-0.007249635	1.485181725	-0.001118275
15519.4	SHELL_RI_BIGGER_THAN_CORE_RI	1.41349698	-0.00140302	1.491101227	0.004801227
73821.6	SHELL_RI_BIGGER_THAN_CORE_RI	1.423117533	-0.004282467	1.502454861	0.016154861
17399.1	SHELL_RI_BIGGER_THAN_CORE_RI	1.41195506	-0.00014494	1.486920173	0.000620173
33676.9	SHELL_RI_BIGGER_THAN_CORE_RI	1.41682311	-0.00207689	1.493692943	0.007392943
12638.1	SHELL_RI_BIGGER_THAN_CORE_RI	1.408062095	-0.006837905	1.484910428	-0.001389572
34894.4	SHELL_RI_BIGGER_THAN_CORE_RI	1.417230152	-0.010169848	1.497244459	0.010944459
13445.1	SHELL_RI_BIGGER_THAN_CORE_RI	1.40638387	-0.00571613	1.478056147	-0.008243853
28907.4	SHELL_RI_BIGGER_THAN_CORE_RI	1.41176678	-0.00713322	1.485281538	-0.001018462

FIG. 71 (Cont.)

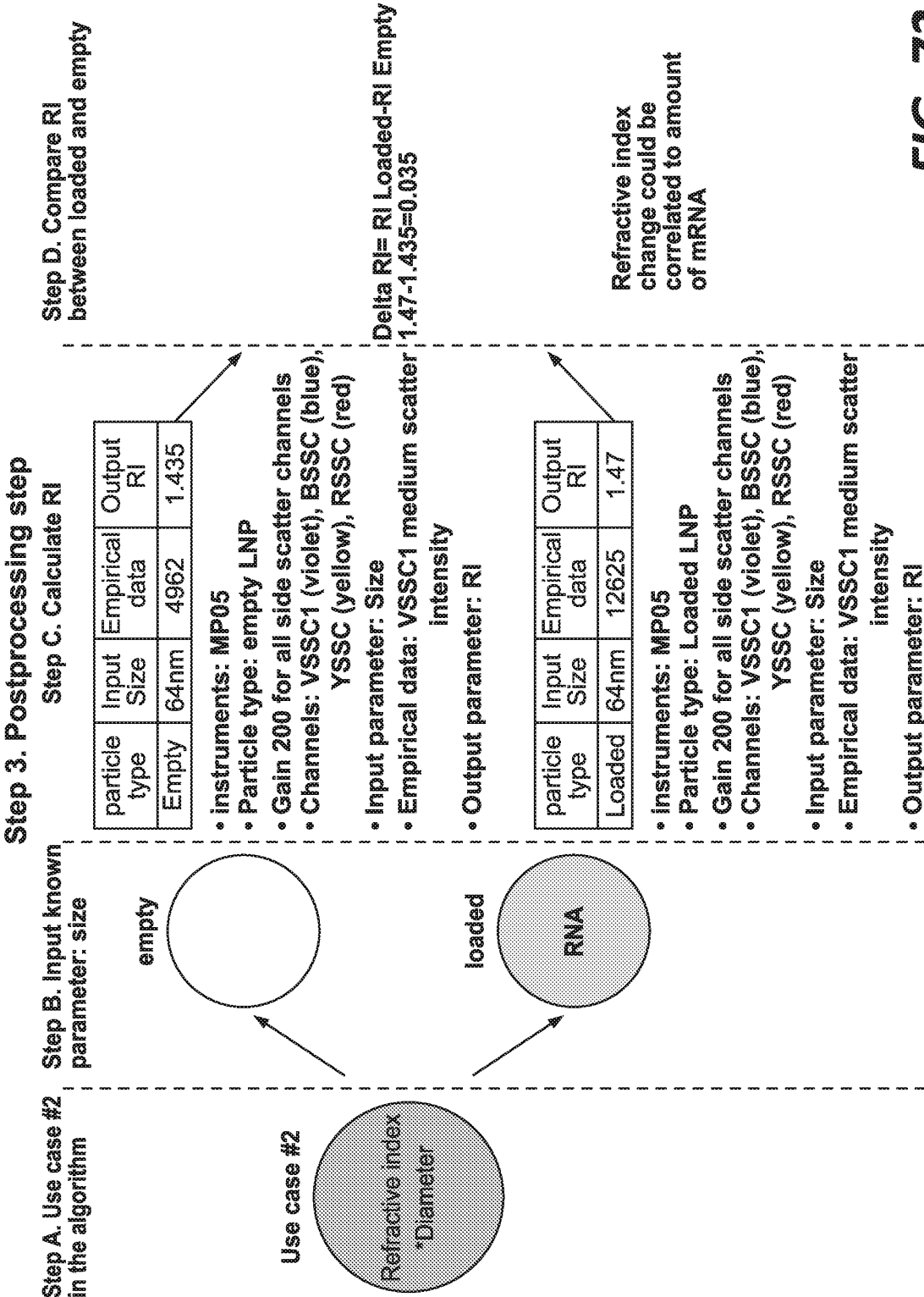


FIG. 72

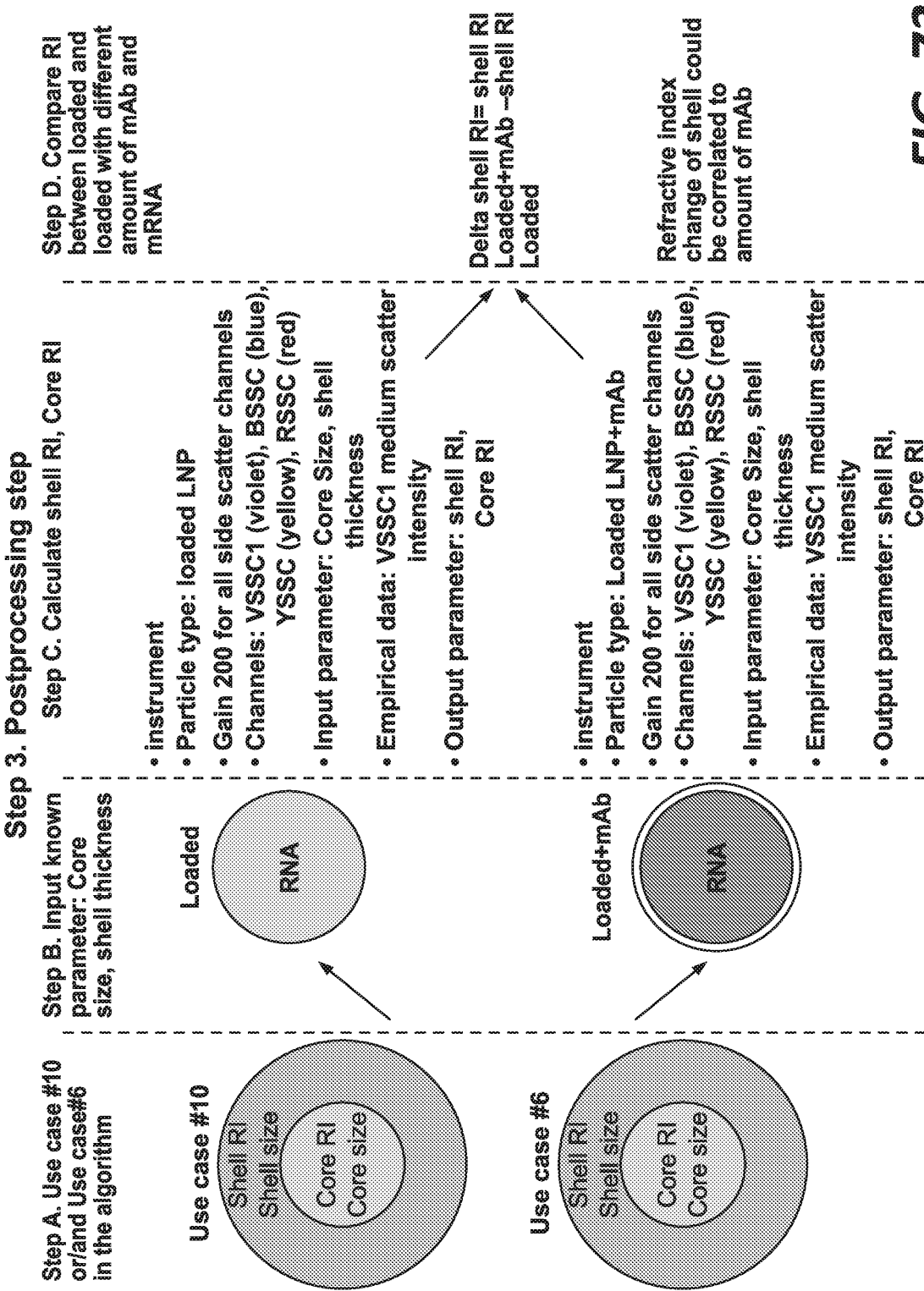


FIG. 73

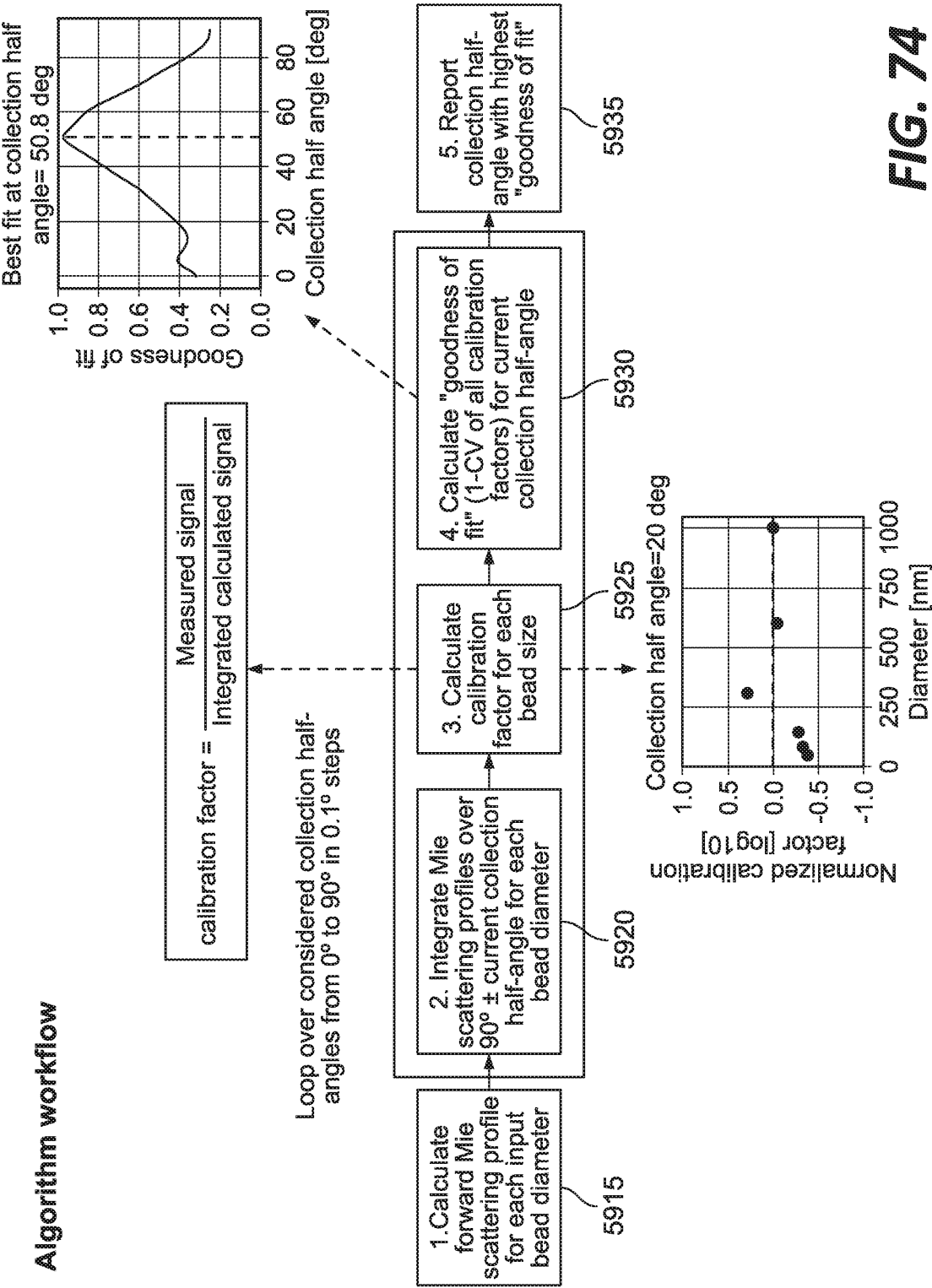


FIG. 74

Step 1: Calculation of the forward Mie scattering profiles

- The pySCATMECH library was used
- Profiles were calculated in the range of 0° to 180° in 0.1° steps
- These profiles do not depend on the collection half-angle, and so are only calculated once
- Inputs:
 - Bead diameter
 - Bead refractive index at wavelength
 - Medium refractive index at wavelength
 - Wavelength
 - Polarization of incident light
 - Detector polarization sensitivity
- Output:
 - Mie scattering intensity as a function of polar angle

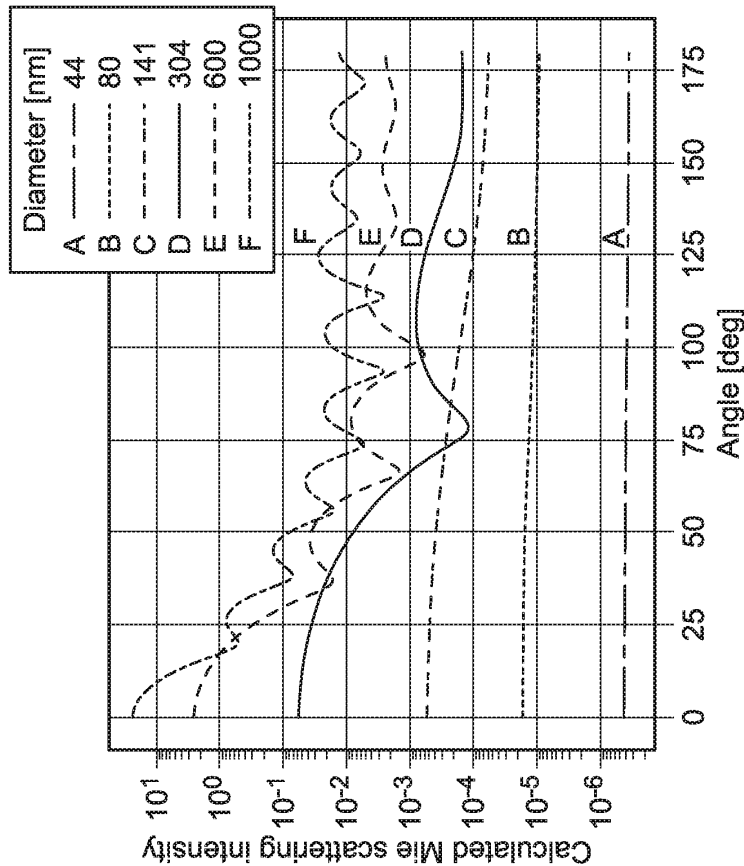


FIG. 75

Step 2: Integrating the calculated scattering intensity

- We used Simpson's rule to numerically integrate the Mie scattering profiles over an angular range that is defined by the current proposed collection angle
- Integrand need to be weighted by $\sin \theta$
- Example outputs are shown on the right

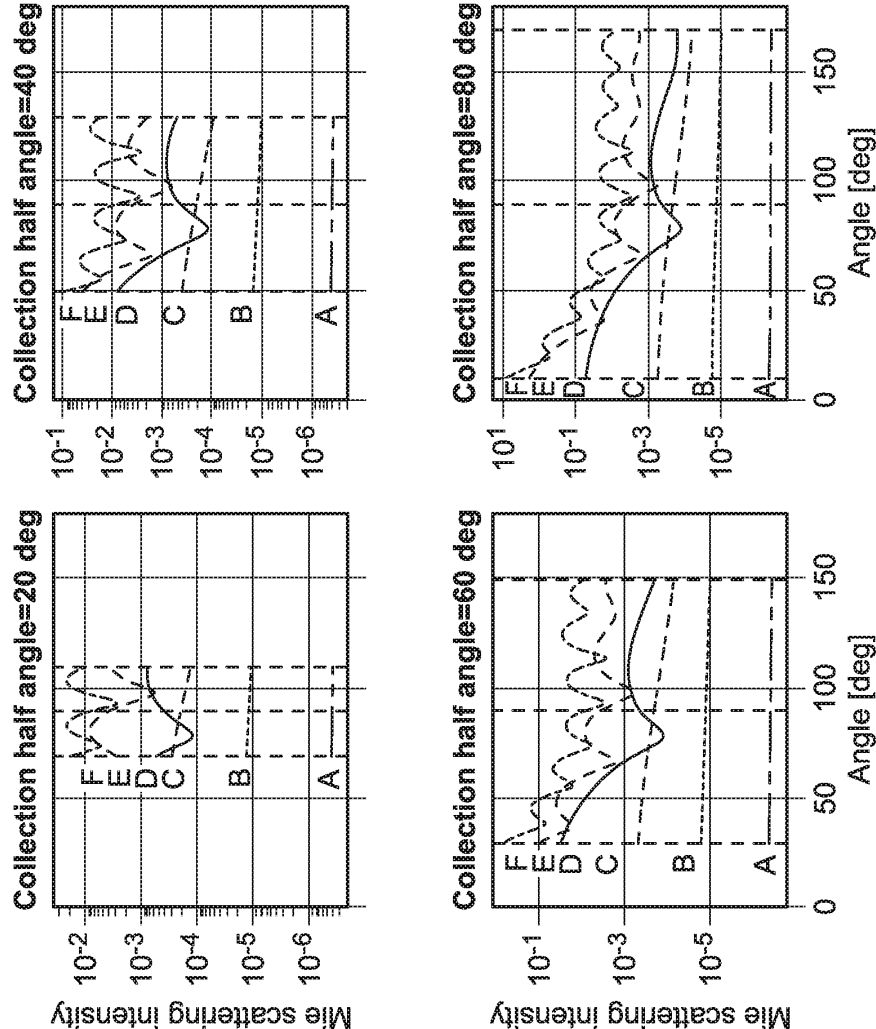


FIG. 76

- Step 3: Calculating the calibration factors
- The calibration factor is defined as the ratio of the measured scattered intensity and the integrated forward Mie intensity that was calculated in Step 2
 - The calibration factor is calculated for each bead diameter that was provided:

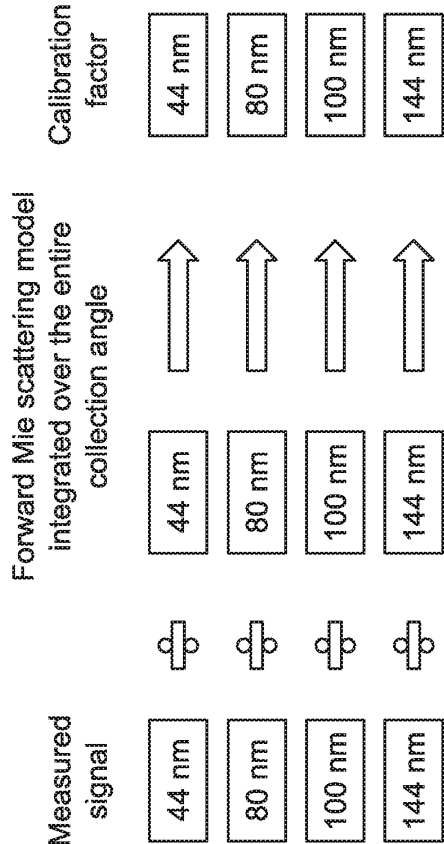


FIG. 77

Step 4: Calculation of the goodness of fit

- The output of Step 3 is a set of calibration factors for each input bead diameter for a single proposed collection half-angle
- First, the Coefficient of Variation (CV) is calculated over the set of calibration factors:

$$CV = \frac{\text{standard deviation(all calibration factors)}}{\text{average(all calibration factors)}}$$

- Second, the "goodness of fit" for the current proposed collection half-angle is calculated:
$$\text{goodness of fit} = 1 - CV$$
- Since $CV \geq 0$, the goodness of fit is ≤ 1 .

FIG. 78

Step 5: Identify the most probable collection angle

- Step 4 is repeated for each collection half-angle that is considered. The output is a "goodness of fit" for each potential collection half-angle
- The calibration factor is an instrument parameter and should not depend on the bead size, so ideally, the CV calculated over multiple bead sizes would be 0. However, in reality there is some bead-to-bead variation due to measurement uncertainty, and so the CV is non-zero
- The most probable collection half-angle is identified as the angle with the smallest CV, since this most closely aligns with the ideal case.
- To calculate it, the collection half-angle with the largest goodness of fit is chosen, since the definition of 1-CV means that this is the angle with the CV closest to 0

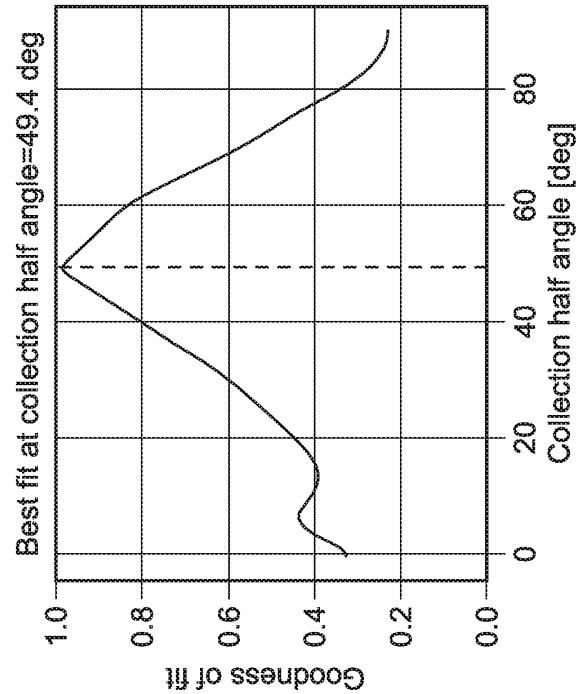
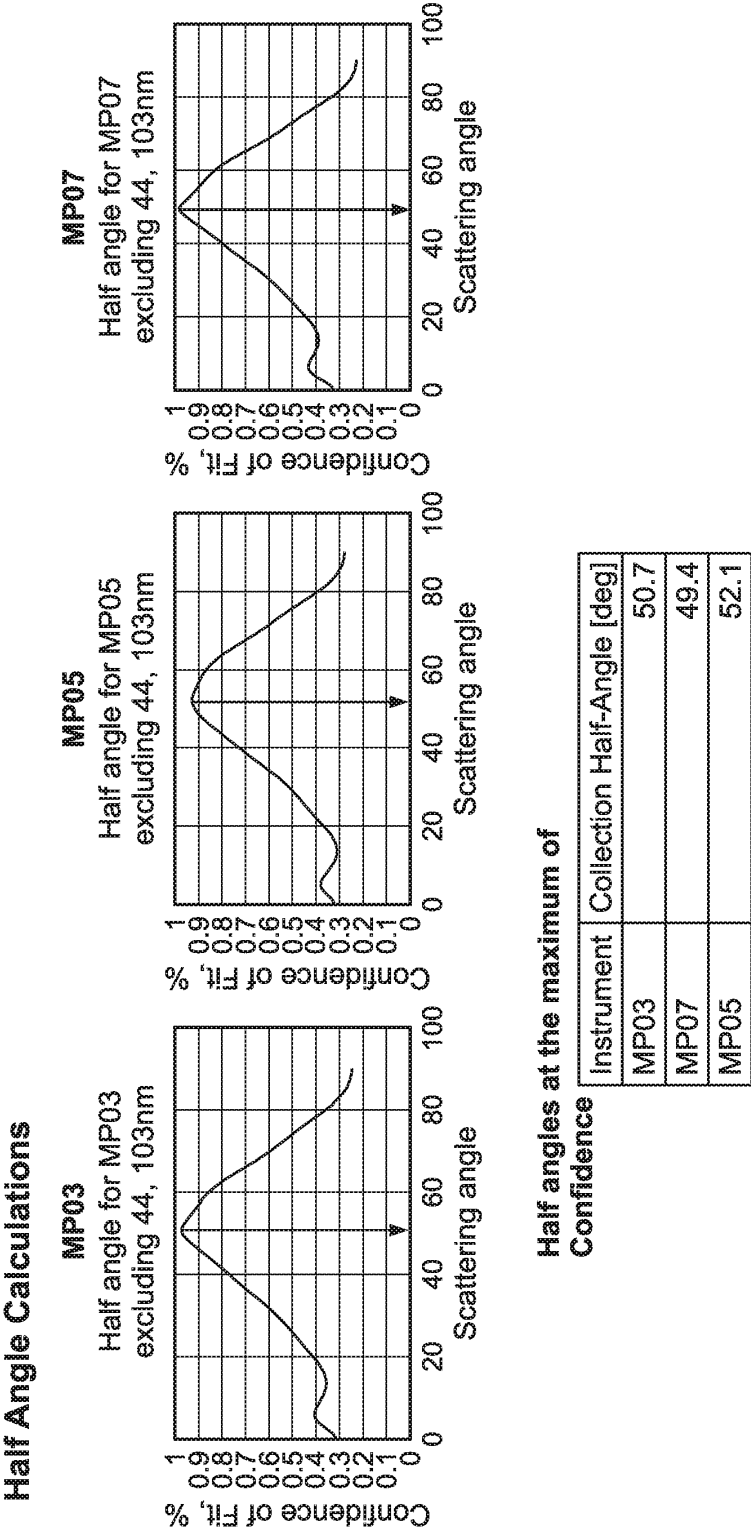
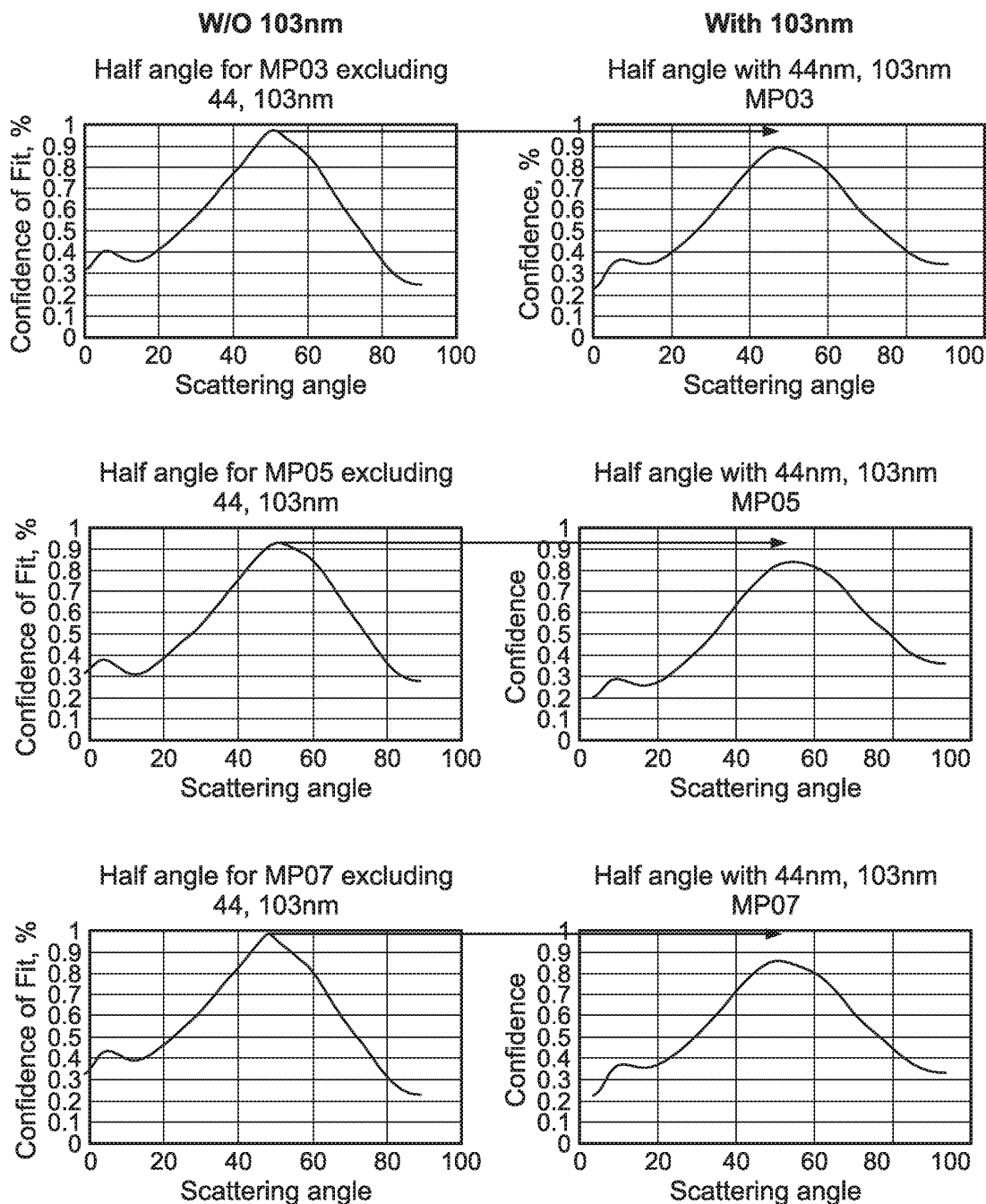


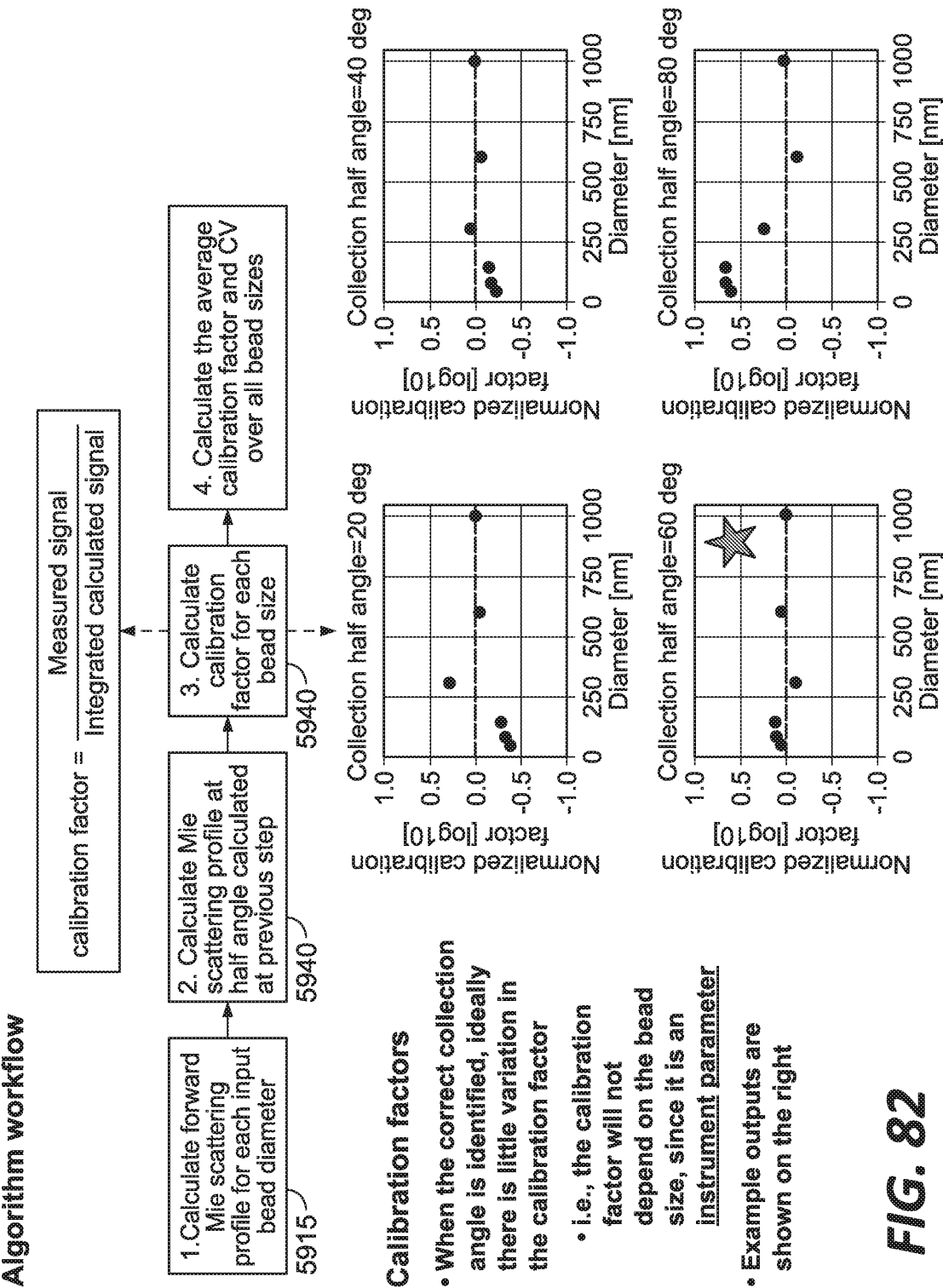
FIG. 79



Half angle is instrument specific but gain independent

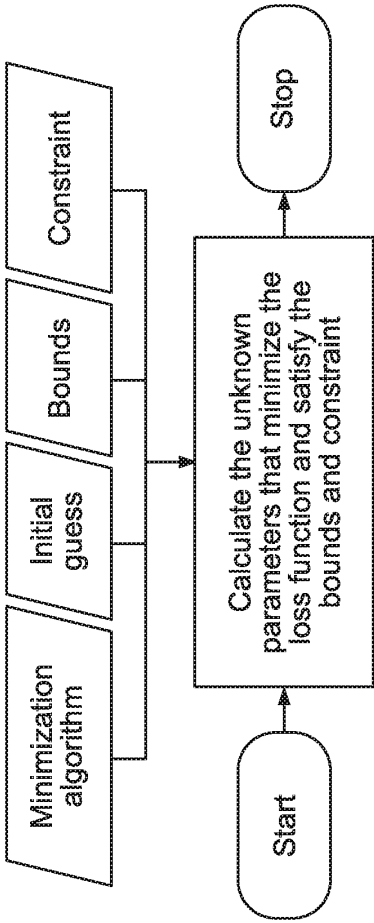
FIG. 80

Comparison analysis of half angle calculations with and without 44nm, 103nm**FIG. 81**



Algorithm

• All use cases use the same fundamental algorithm, with some variation between the parameters of the algorithm



- All use cases use the same loss function:
loss function = |(integrated, calculated Mie signal) x (calibration factor) – measured signal|²
- The calculated Mie signal is a function of the particle parameters listed in the table in Fig. 60

FIG. 83

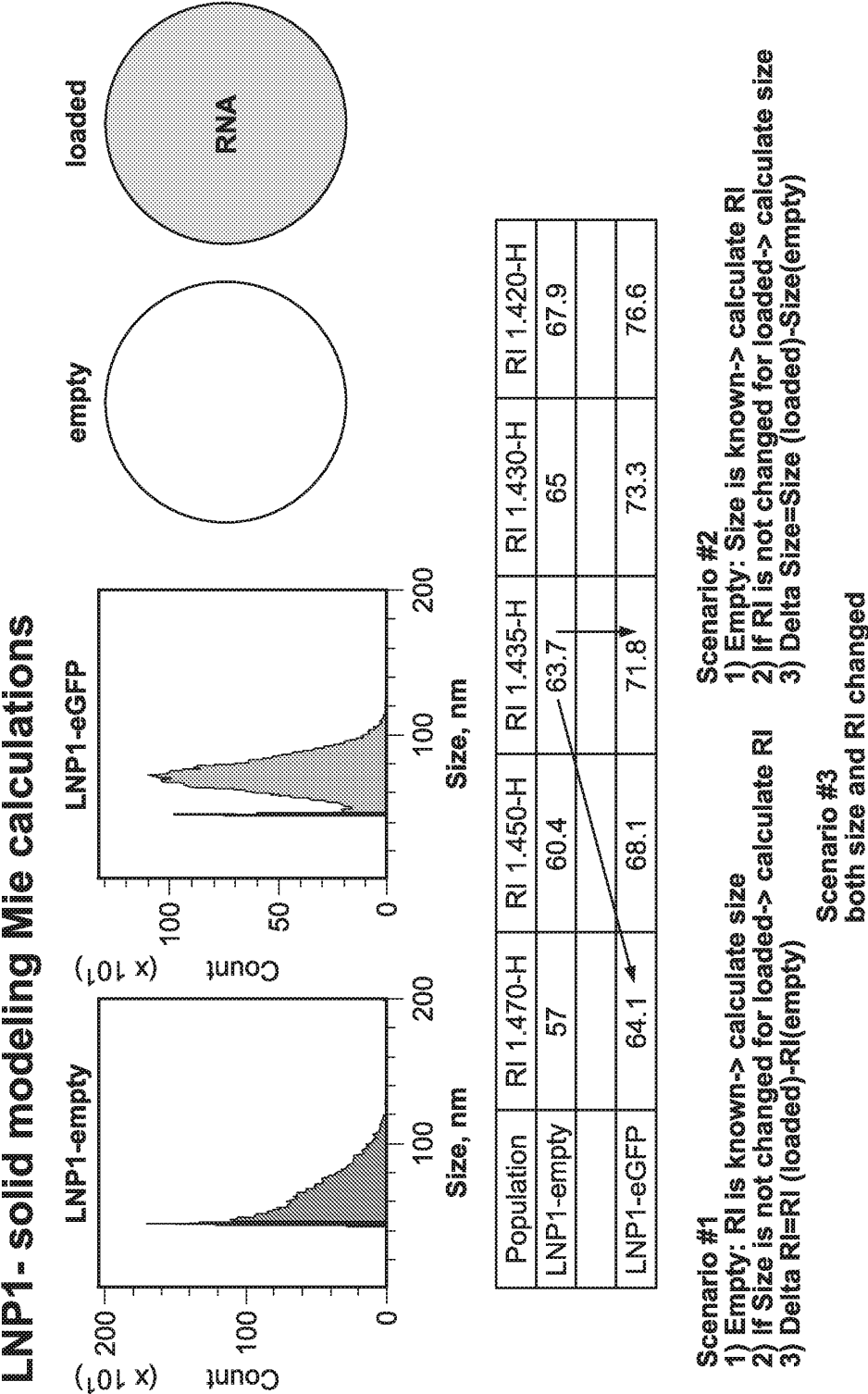


FIG. 84

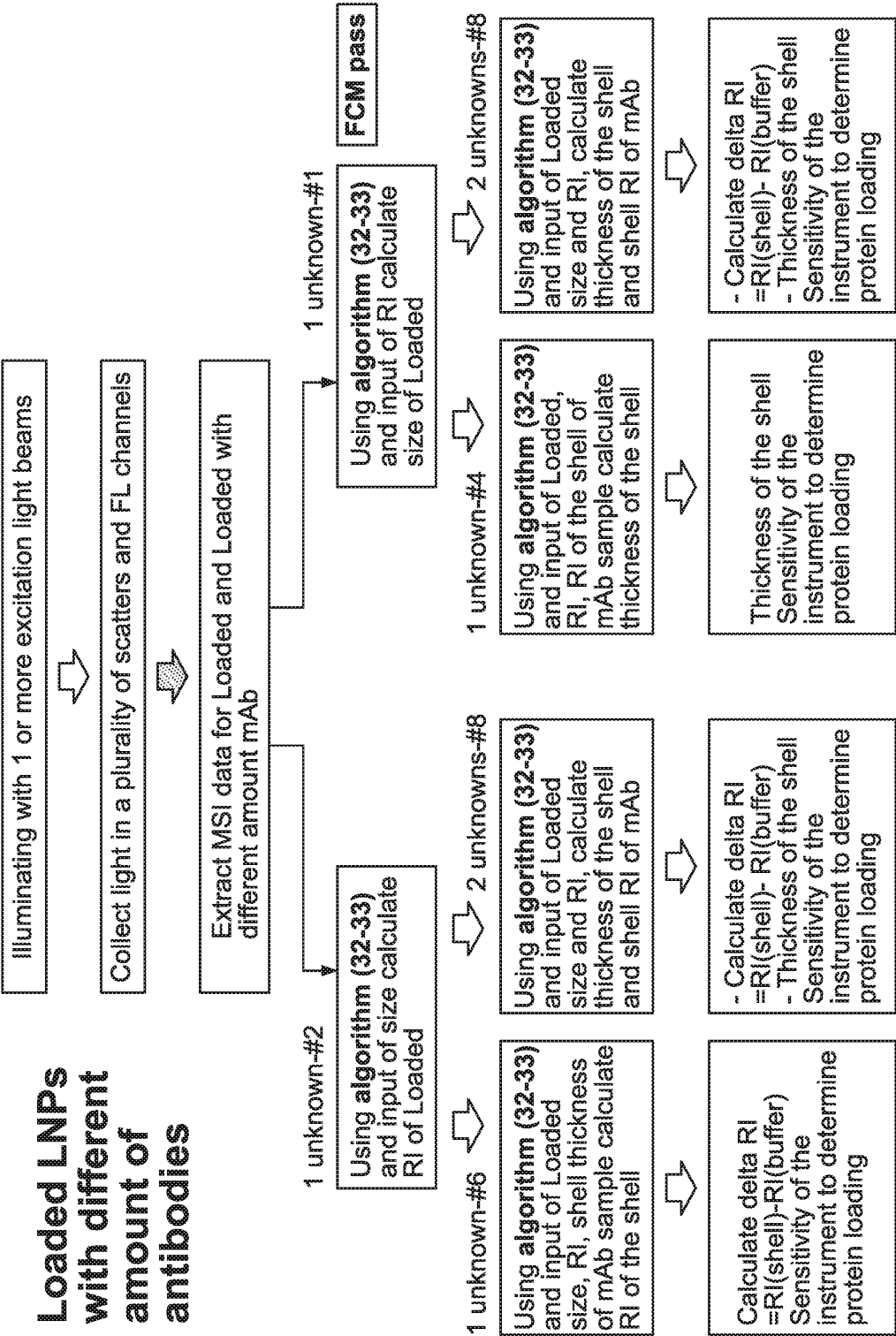


FIG. 85

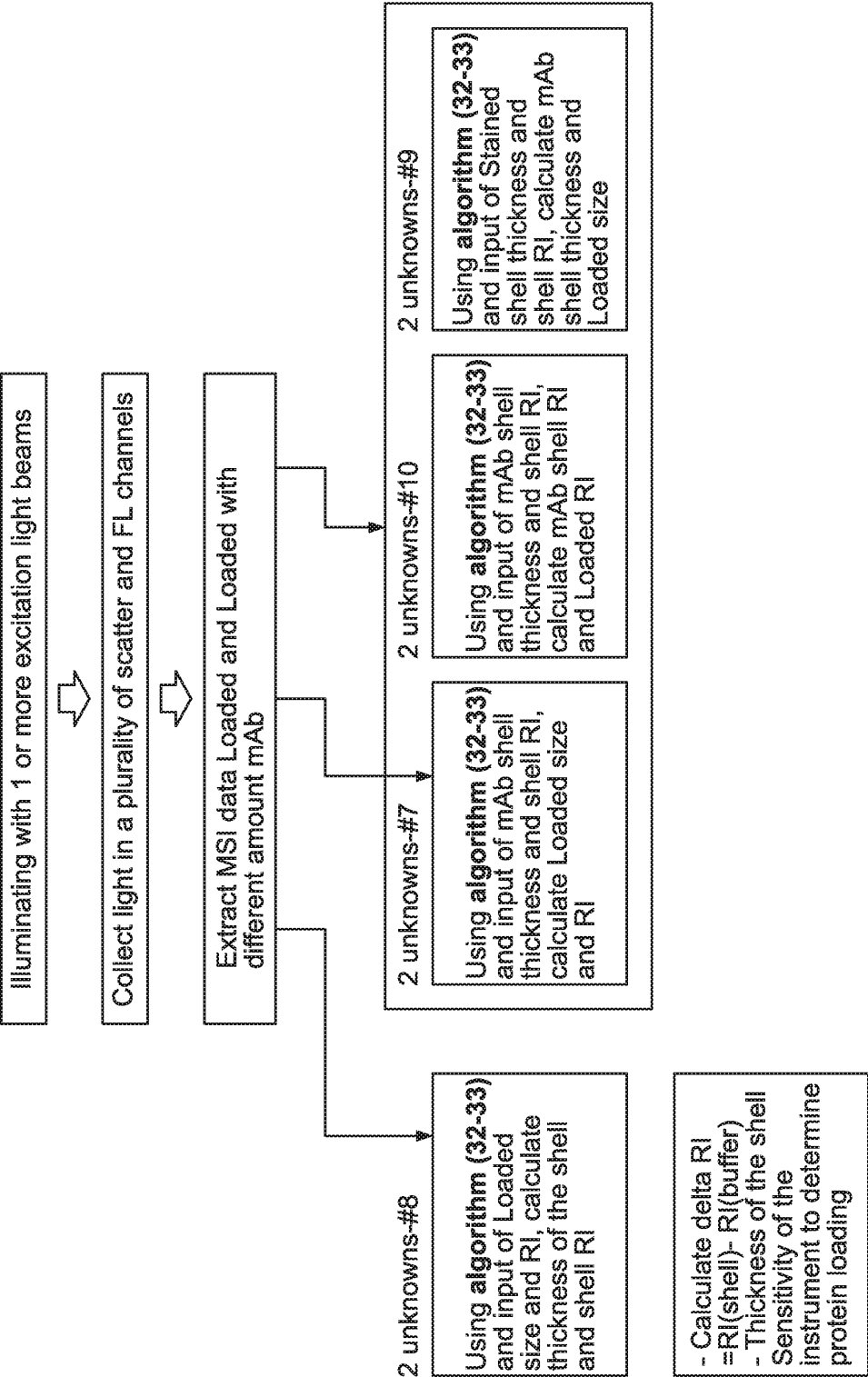


FIG. 86

LNP-LNP1-Raw data

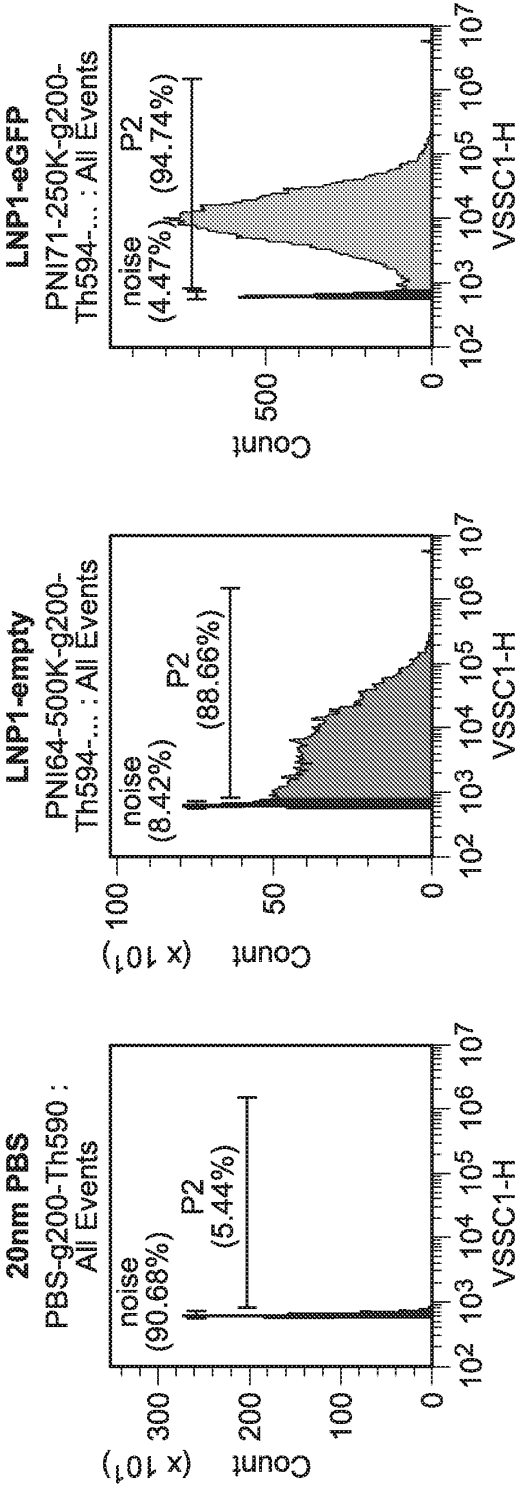


FIG. 87

LNPs-RAW data

Lot #	Description
170001-1	Naked LNP
170001-2	LNP + mAb low
170001-3	LNP + mAb medium
170001-4	LNP + mAb high

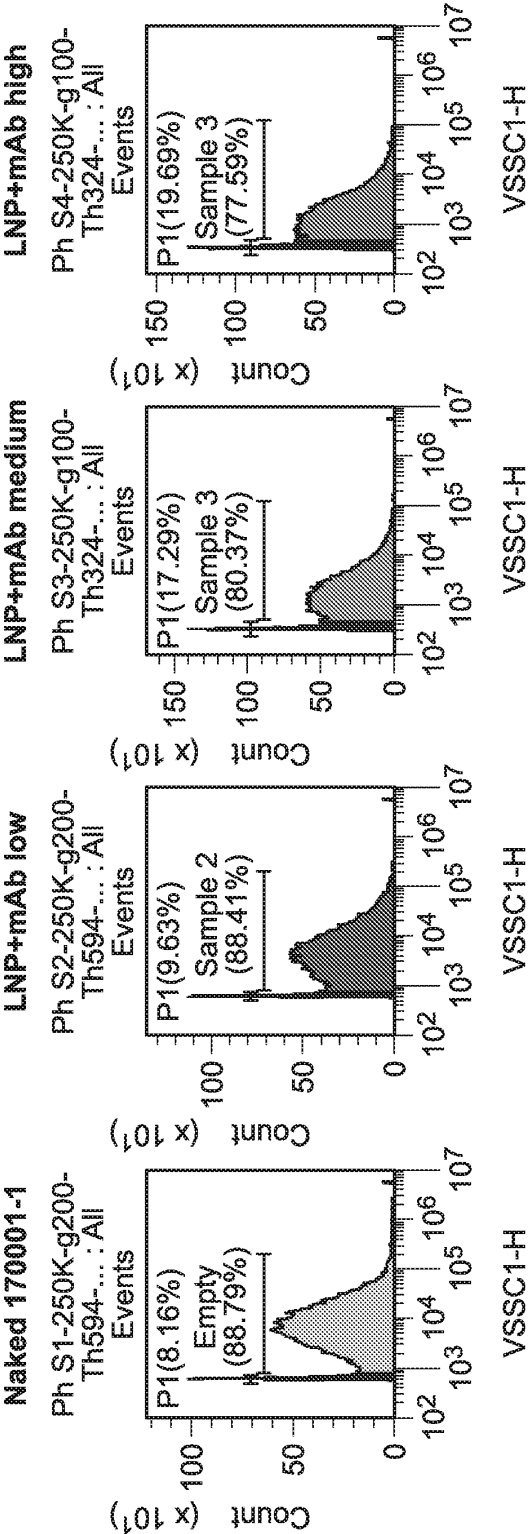
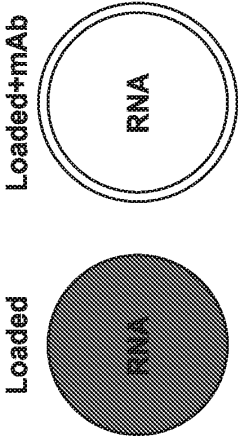


FIG. 88