2020 기초실습 Workshop

[Flowcytometry]

2020.06.20

정 주 연

Flow Cytometry?

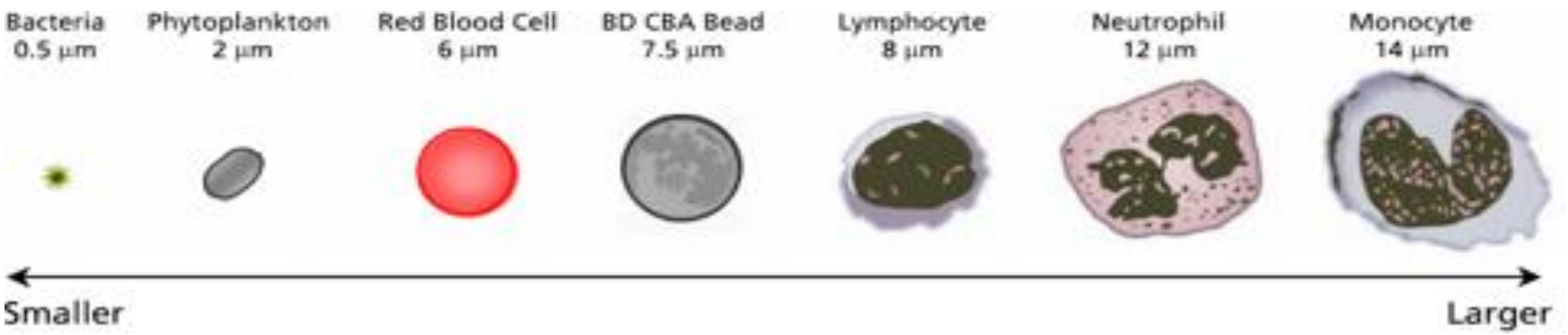
Flow = fluid (흐르다)

Cyto = cells (세포)

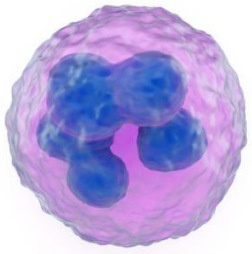
metry = measurement (측정)

: 세포를 유액(Fluid)상태로 흘려 측정하는 방법

FACS : Fluorescence-activated cell sorting



What does it measure?



1. 상대적인 크기 (Relative size)

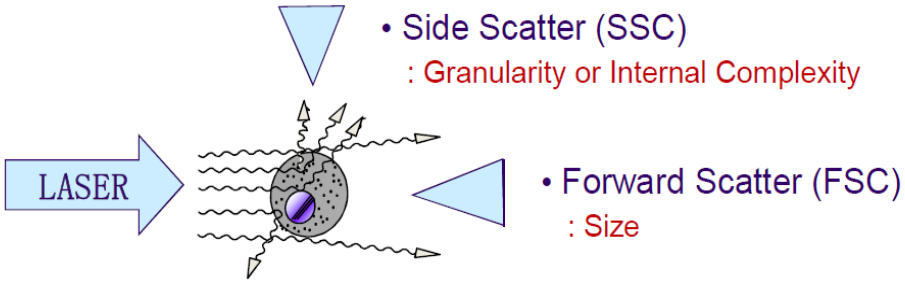
FSC

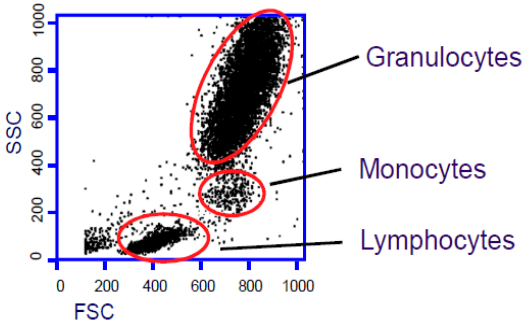
2. 상대적인 내부 복잡성 (Relative internal complexity)

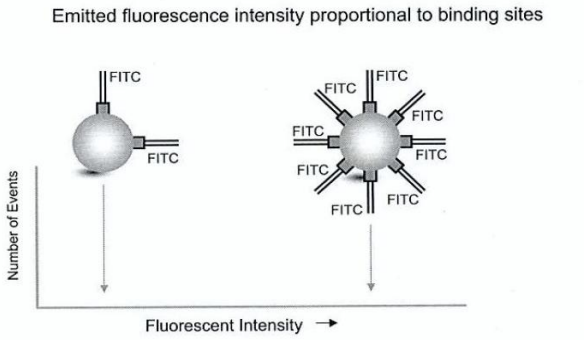
SSC

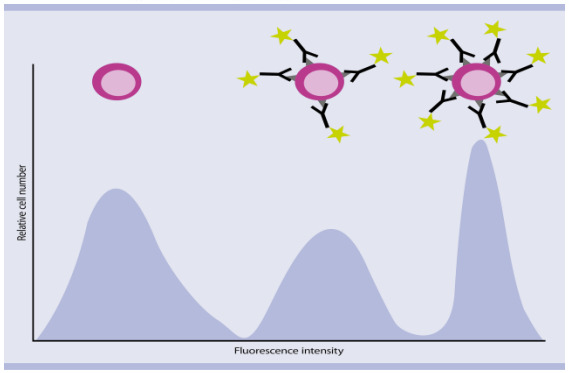
3. 상대적인 형광강도 (Relative Fluorescence intensity)

FL







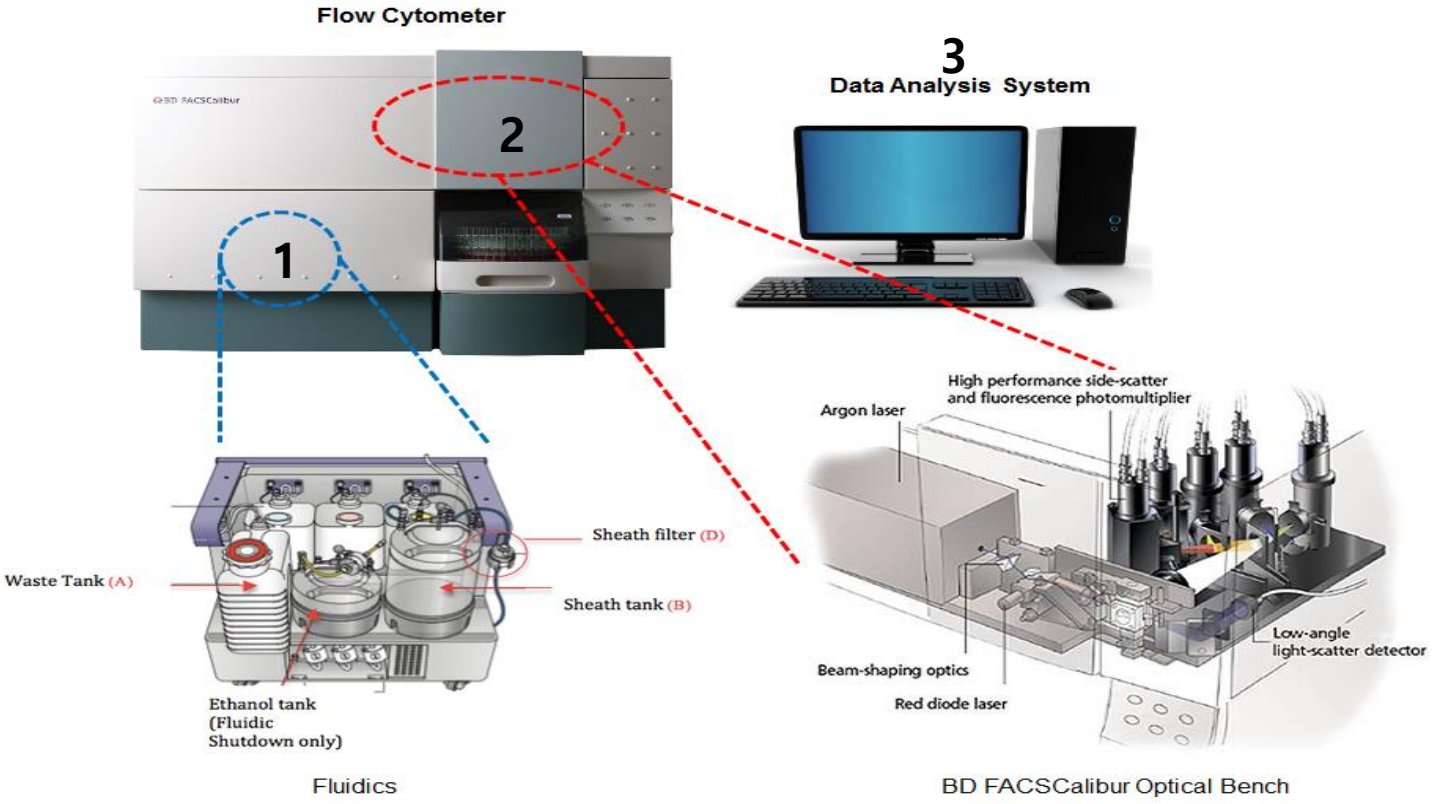


Flowcytometer Subsystem

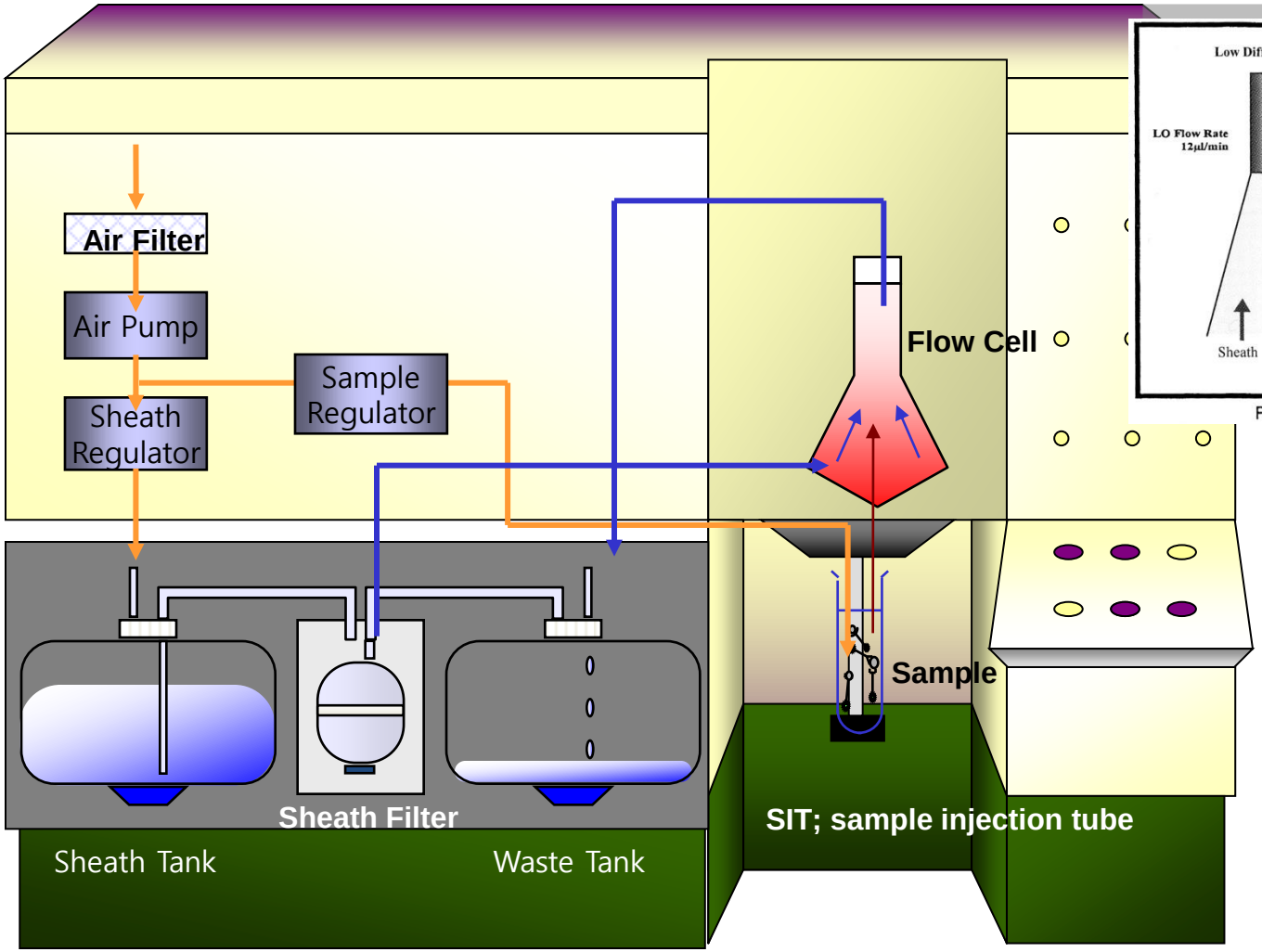
1. Fluidics : Cells in suspension flow

2. Optics : Light and fluorescence is collected, filtered and converted

3. Electronics : Light signals were converted to digital values that are stored in a computer



1. Fluidic System



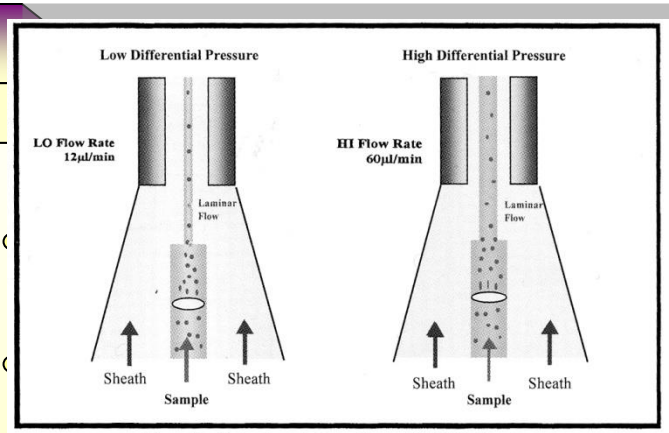
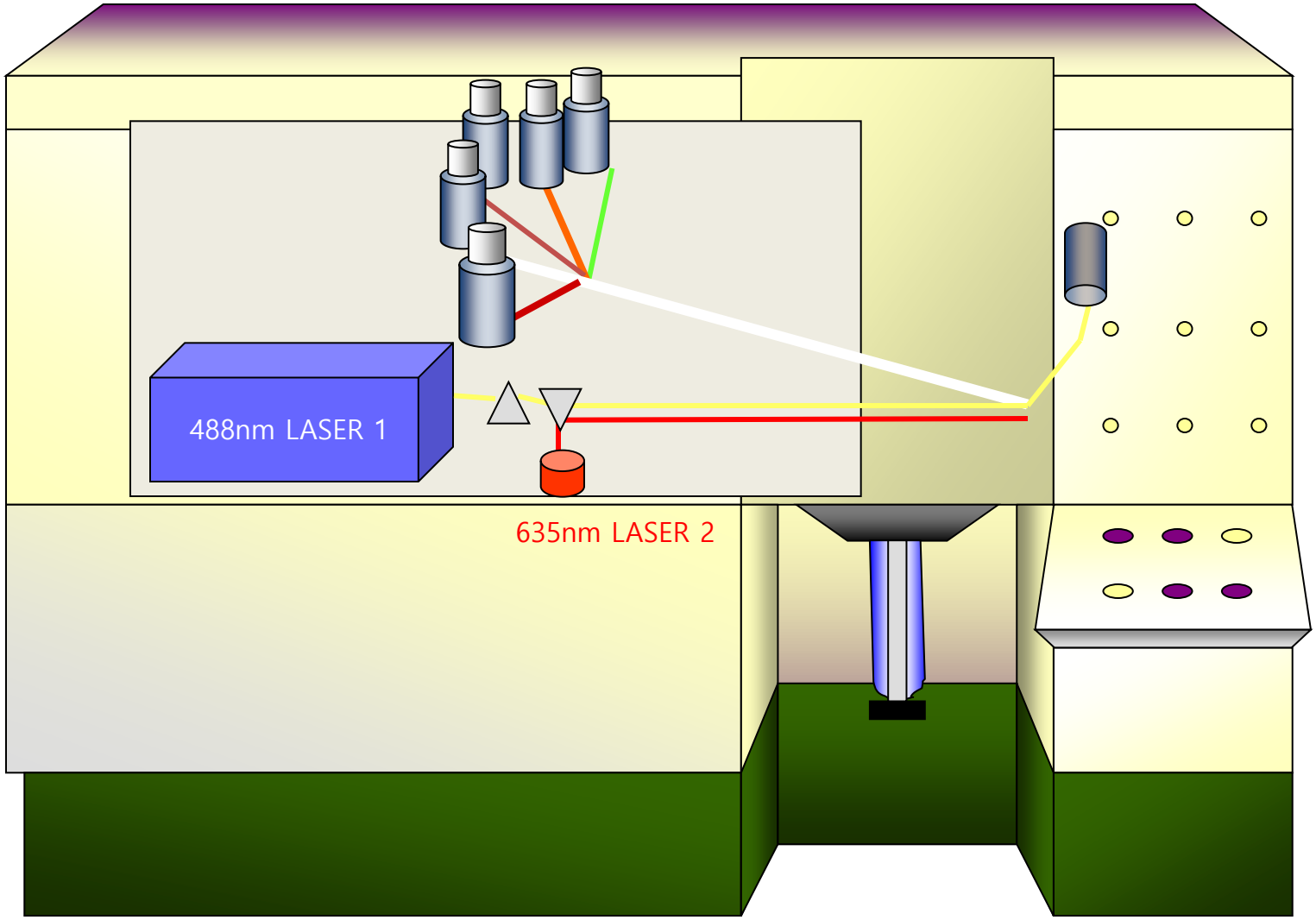


Figure 2 : Hydrodynamic focussing and FACSCalibur flow area

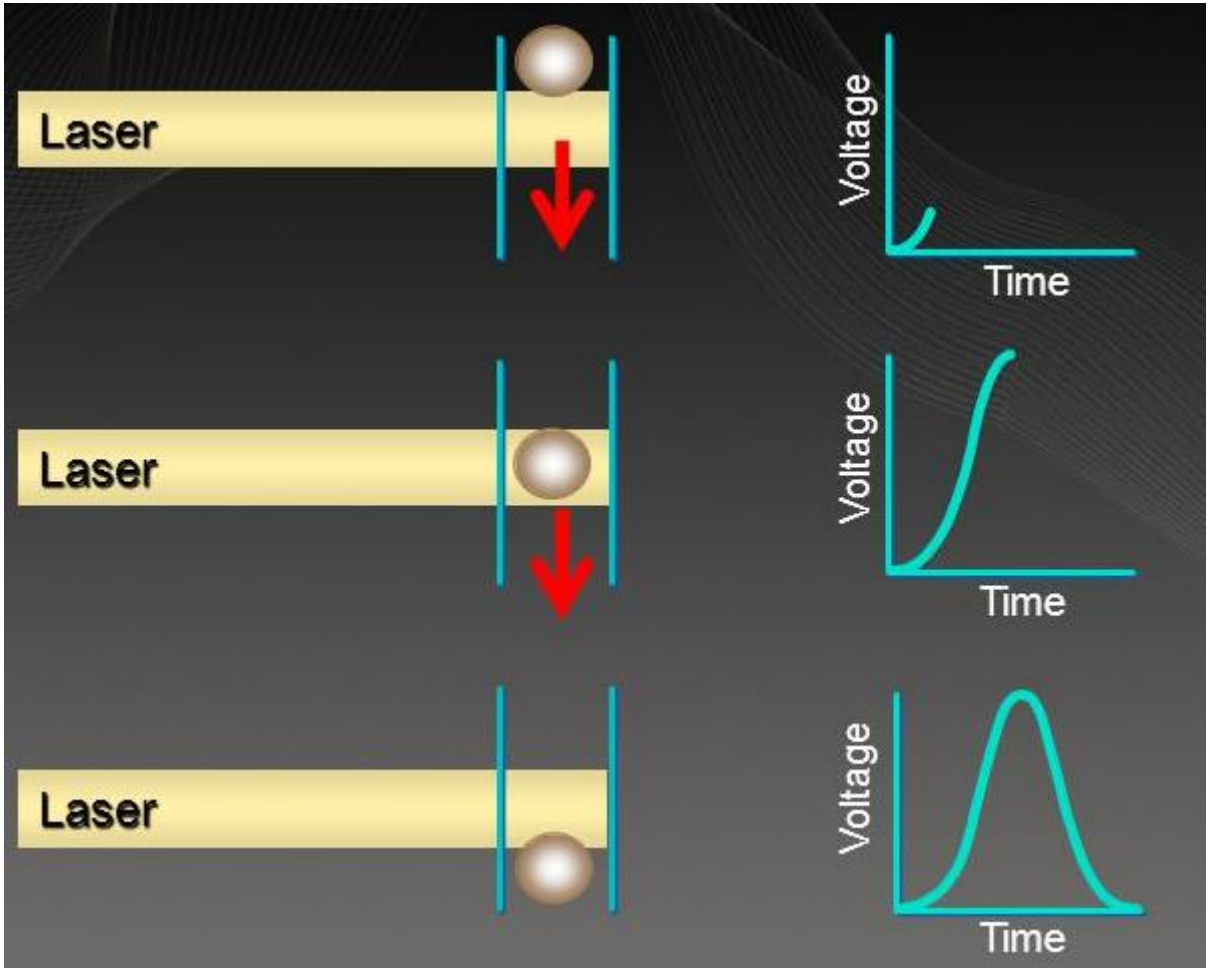
BD
BD Biosciences

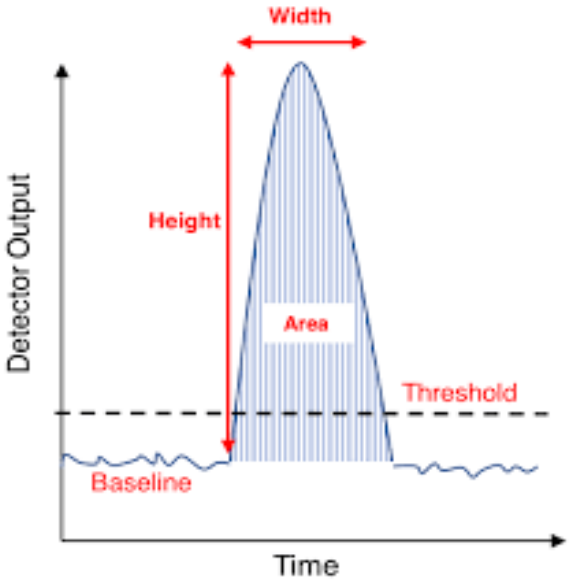
2. Optics System



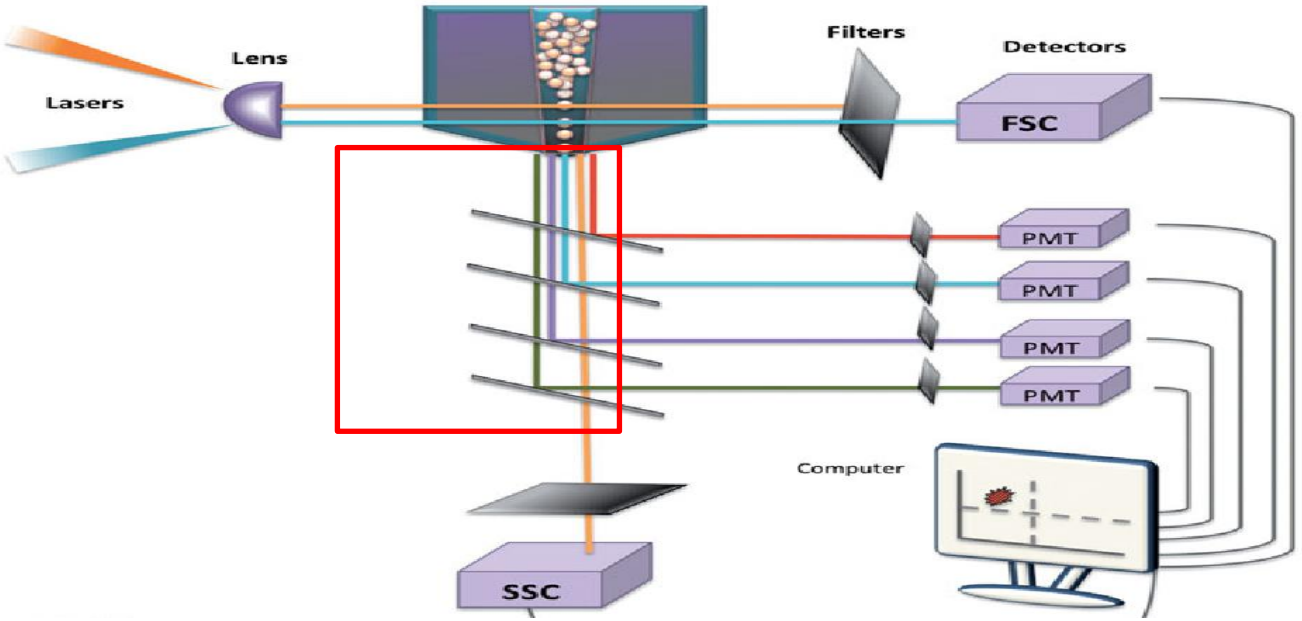
BD
BD Biosciences

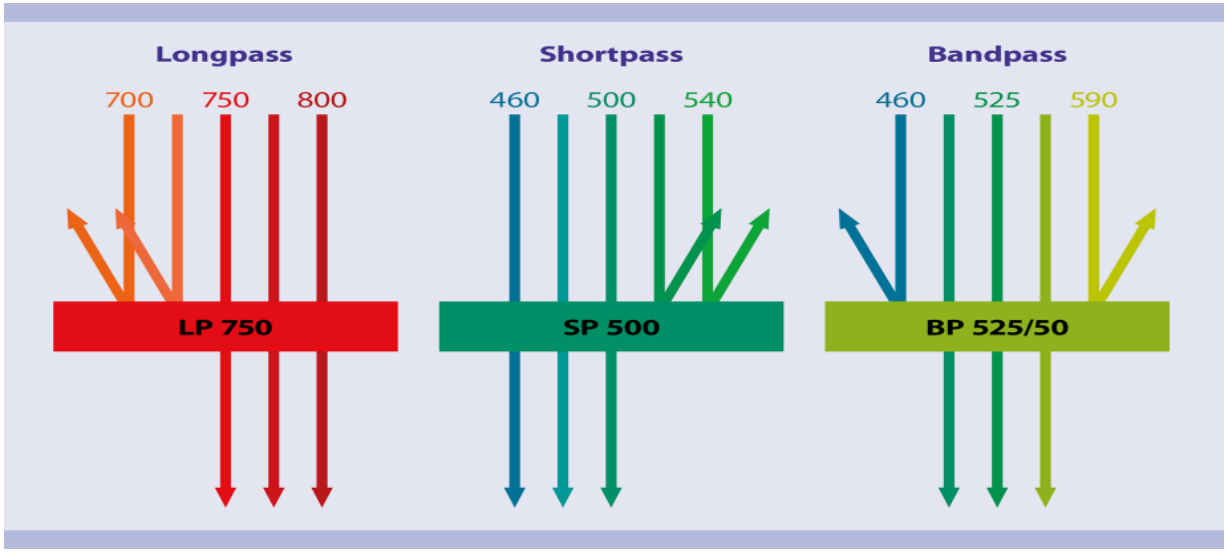
3. Electronics System



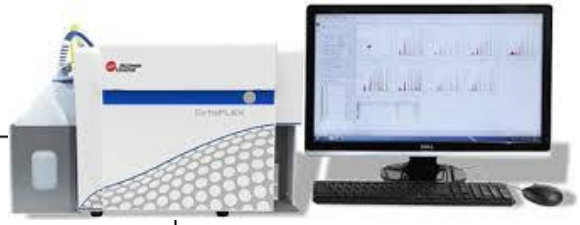


Types of Optical Filters





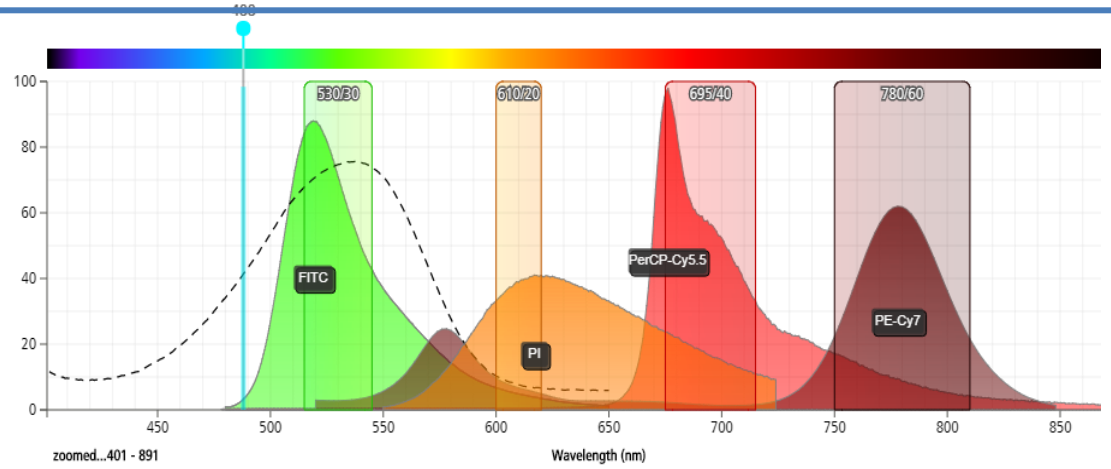
Bandpass filter & Fluorescence dye

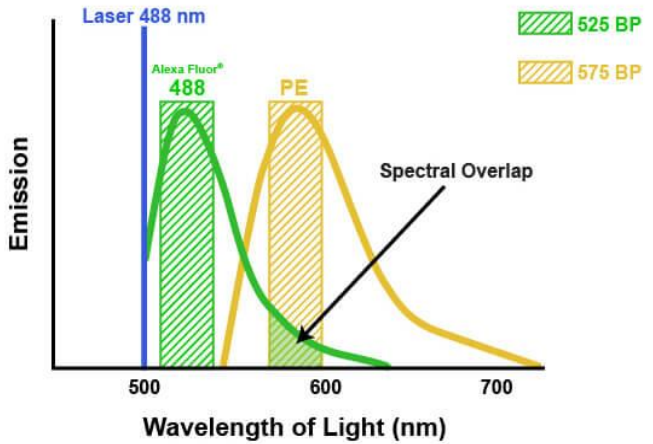


형광 채널(Bandpass Filter) & 형광 dye 표

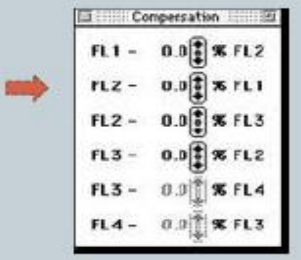
레이저	형광 채널 (badpass filter)	Configuration 표시 이름	사용가능한 형광 이름
488nm	525/40 BP	FITC	FITC, Alexa Fluor 488, GFP, CFSE, Fluo-3
	585/42 BP	PE	PE, PI
	610/20 BP	ECD	ECD, PE-Texas Red, PE-CF594, PI
	690/50 BP	PC5.5	PC5.5, PC5, PE-Cy5.5, PE-Cy5, PerCP, PerCP-Cy5.5, PI
	780/60 BP	PC7	PC7, PE-Cy7
638nm	660/20 BP	APC	APC, Alexa Fluor 647, eFluor 660
	712/25 BP	APC-A700	APC-A700, Alexa Fluor 700
	780/60 BP	APC-A750	APC-A750, APC-Cy7, APC-H7, APC-eFluor 780
405nm	450/45 BP	PB450	Pacific Blue, V450, eFluor 450, BV421
	525/40 BP	KO525	Krome Orange, AmCyan, V500, BV510
	610/20 BP	Violet610	BV605, Qdot 605
	660/20 BP	Violet660	BV650, Qdot 655
	780/60 BP	Violet780	BV785, Qdot 800

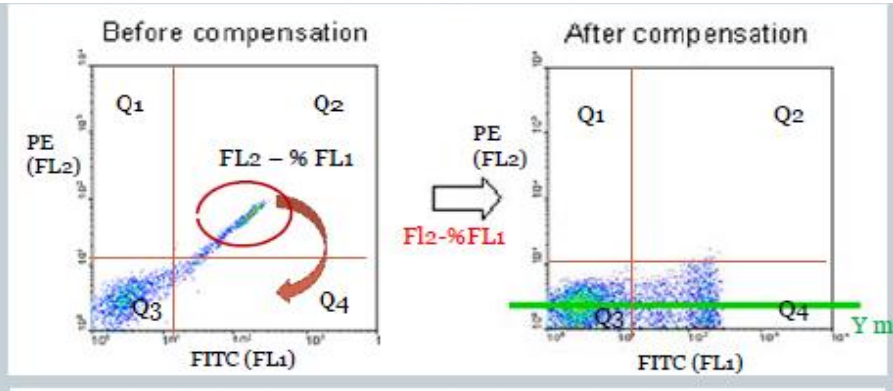
Compensation





Cells + FITC

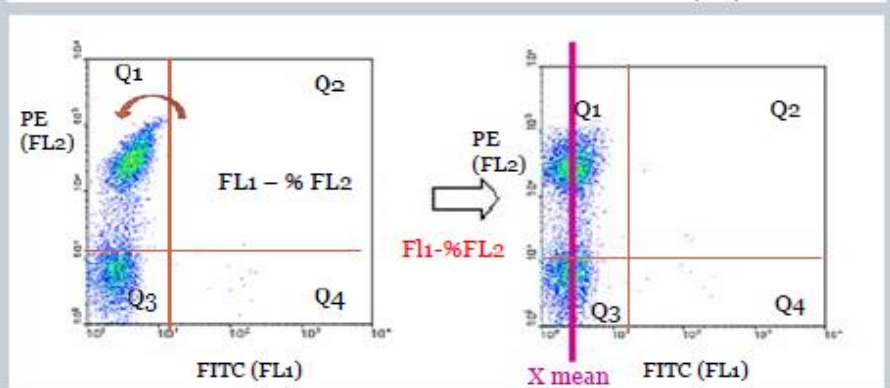




Quadrant Statistics

Quad	Events	X Mean	Y mean
Q1	0	***	***
Q2	0	***	***
Q3	7198	3.87	3.27
Q4	2786	336.3	3.32

Cells + PE

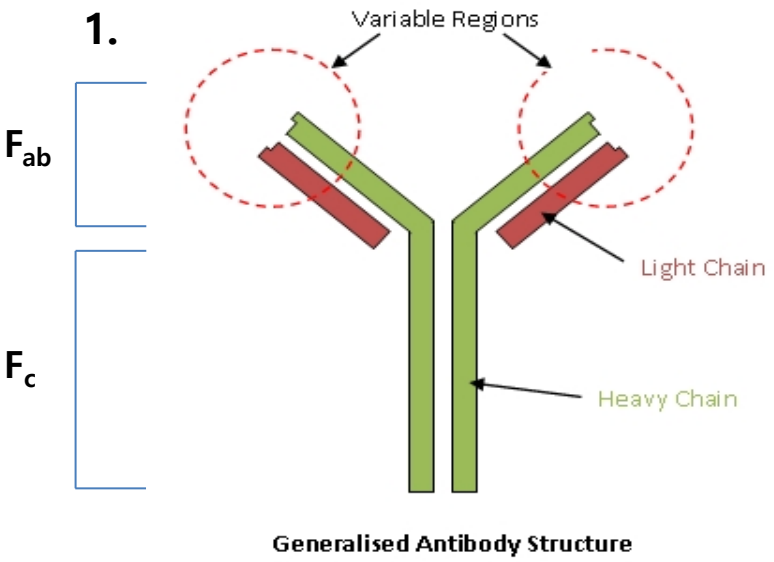


Quadrant Statistics

Quad	Events	X Mean	Y mean
Q1	318	3.51	600.71
Q2	0	***	***
Q3	1035	3.86	4.03
Q4	0	***	***

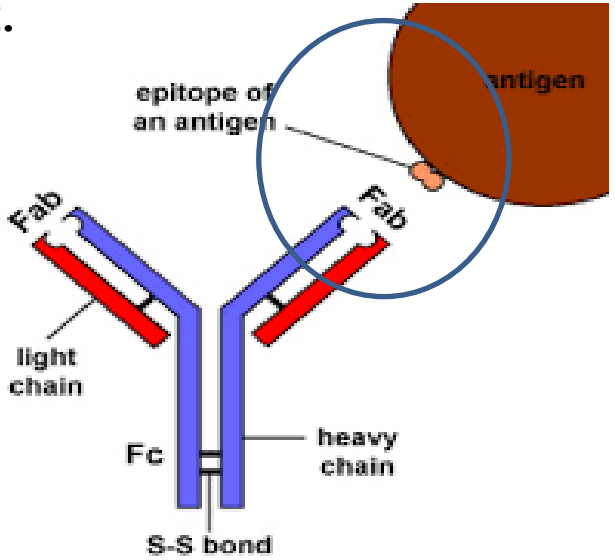
Antibody (항체)

1.

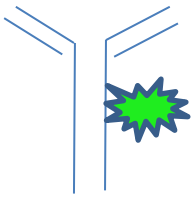


Generalised Antibody Structure

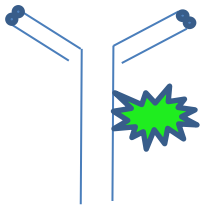
2.



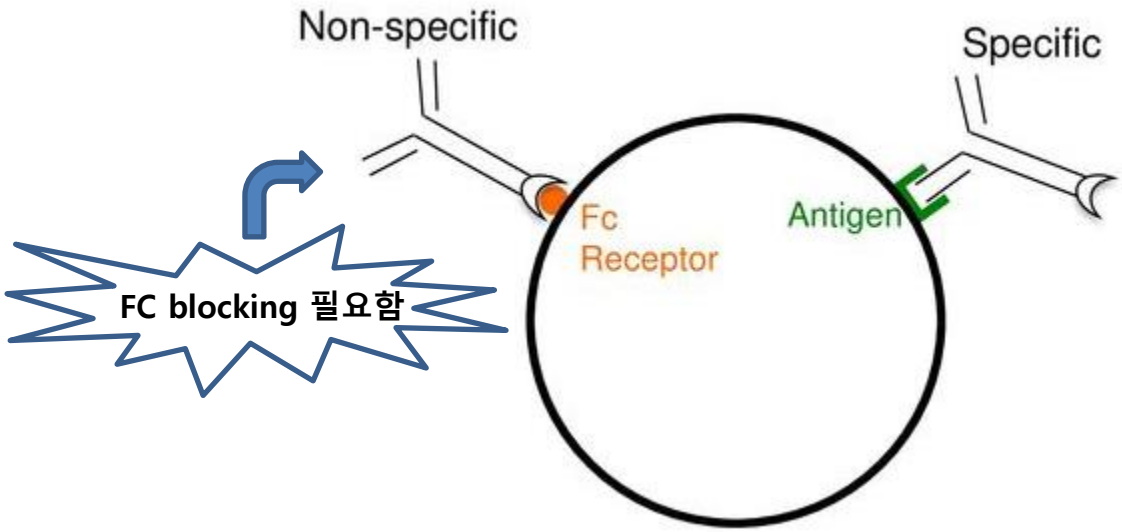
3. Isotype antibody



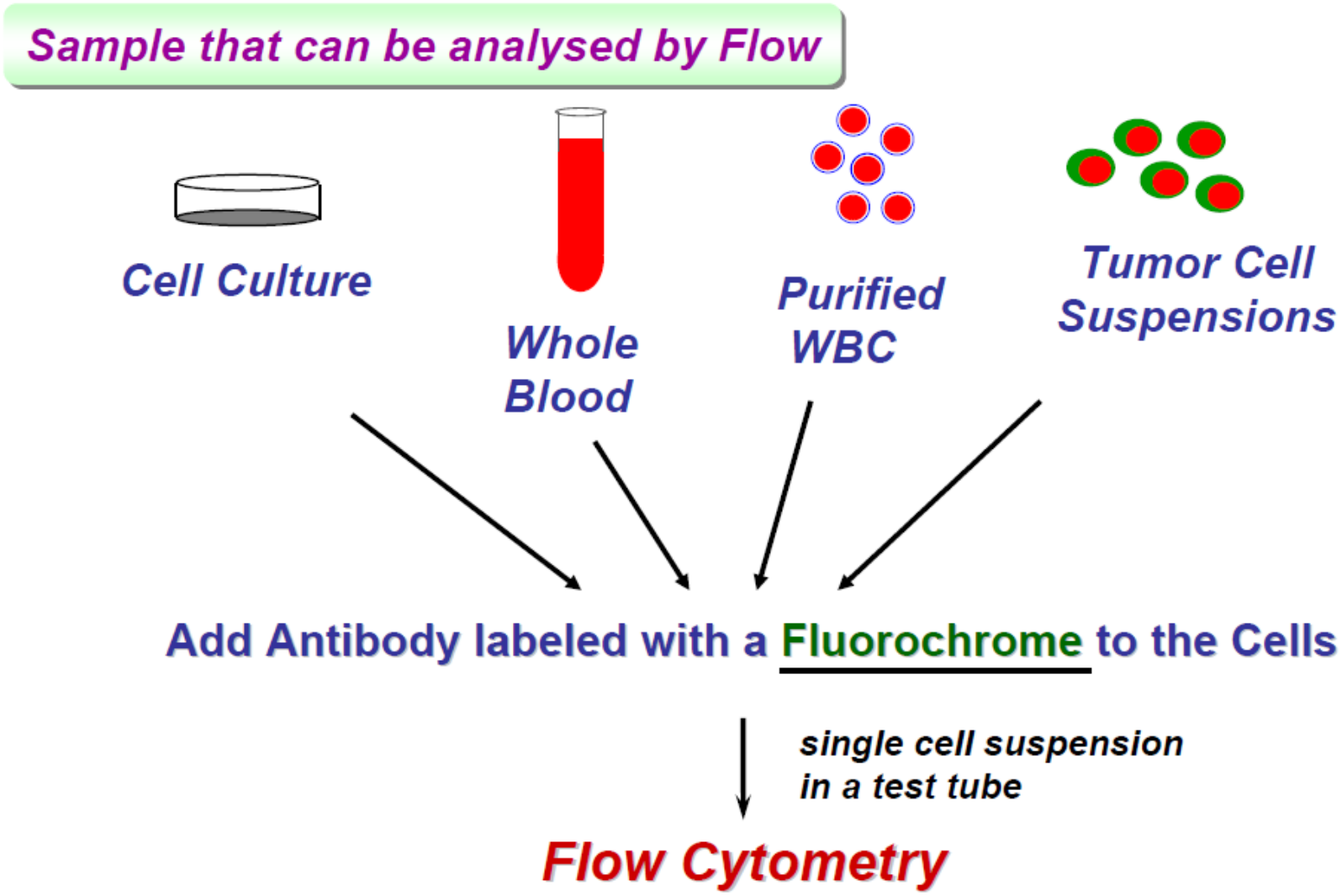
Target antibody



4.

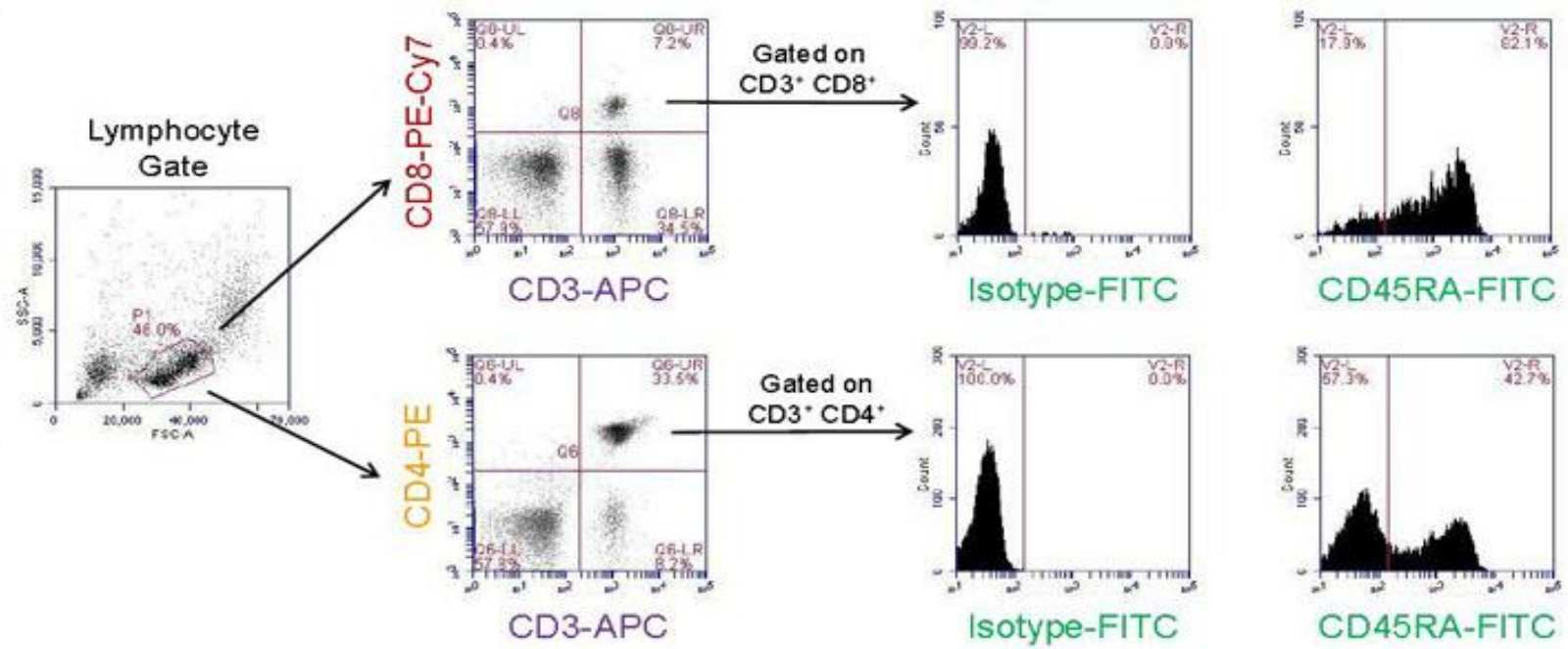


Sample prepararation

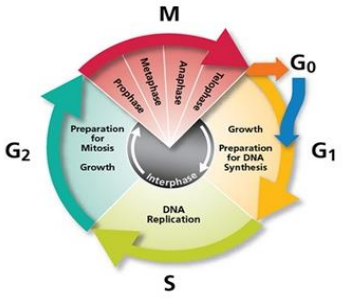


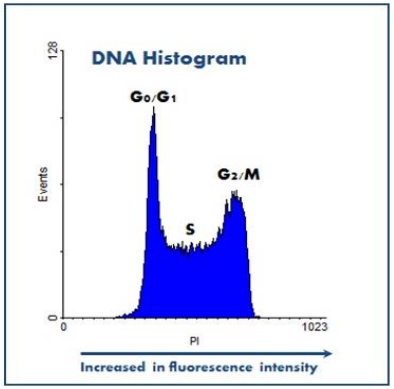
Application of Flow Cytometry

Immunophenotyping

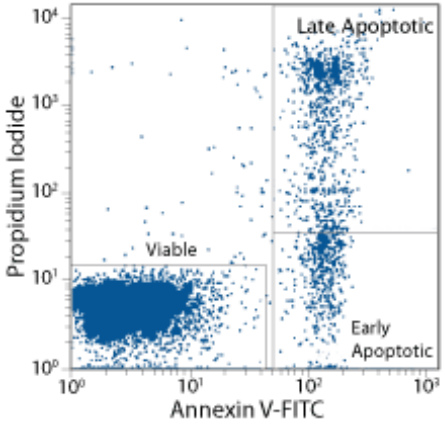


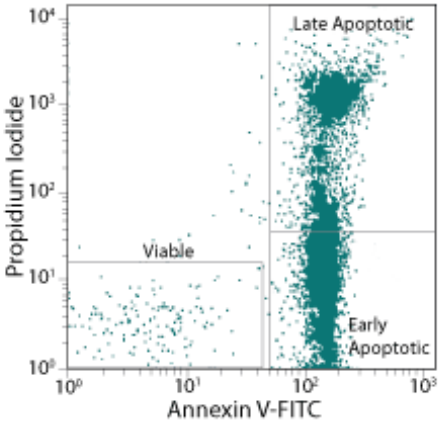
Cell Cycle Analysis



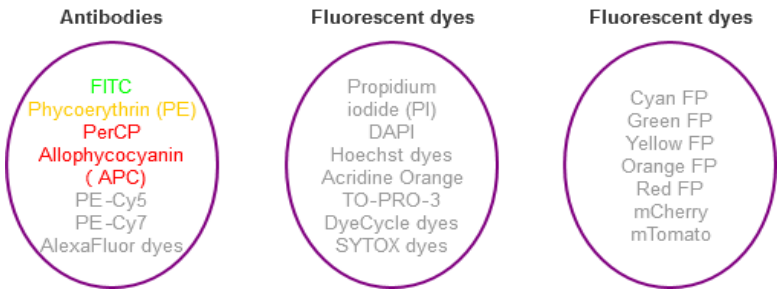


Apoptosis Studies



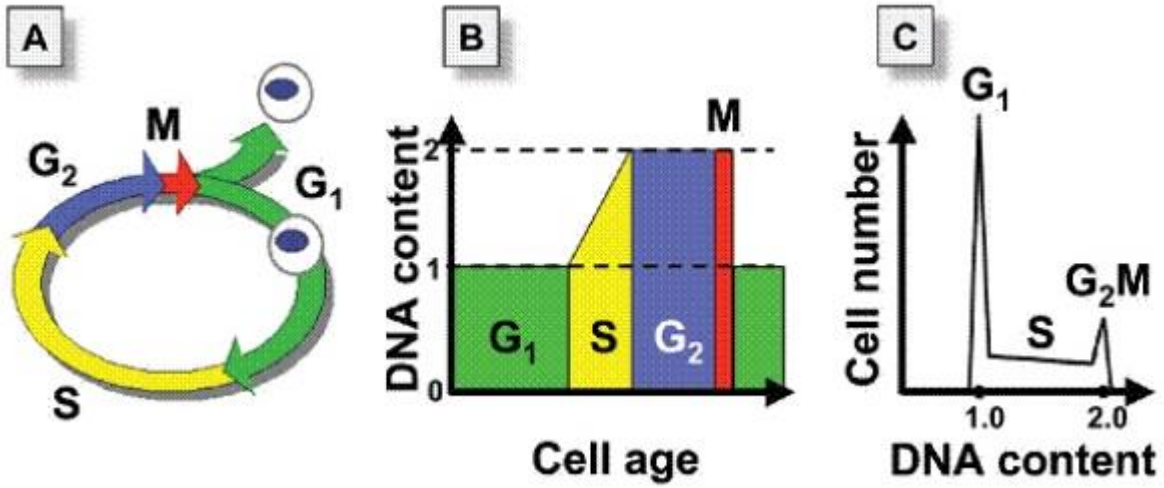


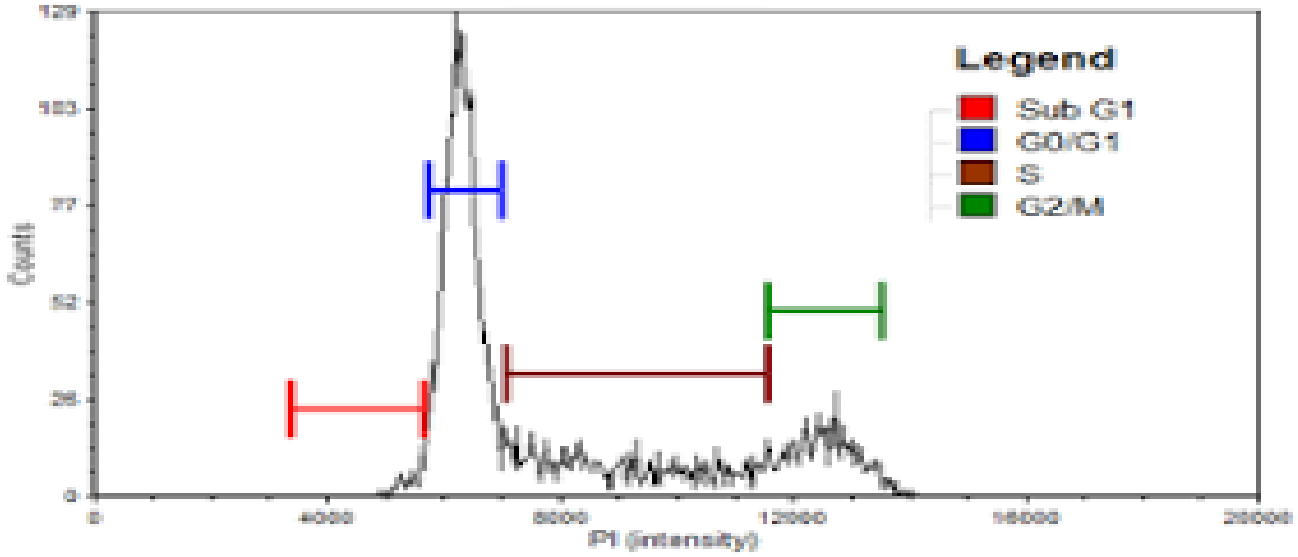
Application of Flow Cytometry



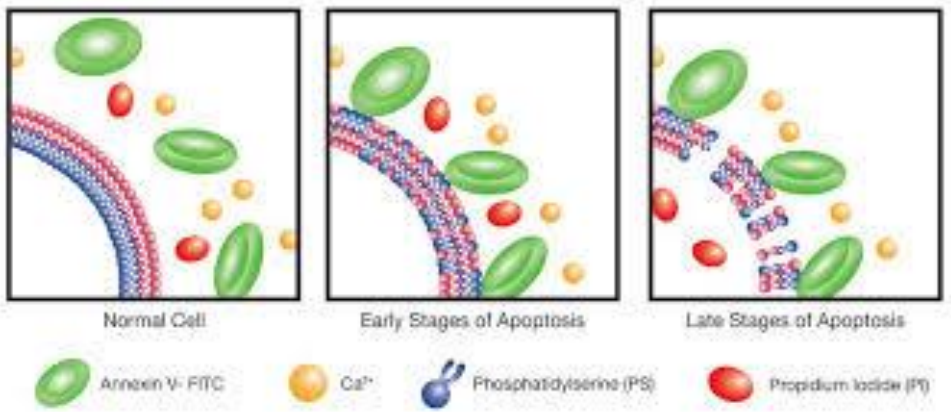
- ✓ **Surface** phenotyping : Ab against cell surface Ag, MHC tetramer *etc*
- ✓ Staining of **intracellular** protein after cell permeabilization
- ✓ ICS (intracellular cytokine staining) with brefeldin A/monensin
- ✓ Detection of **phosphorylated** protein
- ✓ Cell **proliferation** (CFSE, BrdU *etc)*
- ✓ Analysis of intracellular **ion** concentration
- ✓ Analysis of reduction/oxidation (**redox**) potential
- ✓ **Cell cycle** analysis (by using DNA-binding dye)
- ✓ **Apoptosis** analysis (annexin V, TUNEL *etc)*
- ✓ **Cytokine secretion** assay (& subsequent isolation)
- ✓ **CBA** (cytometric bead array) - Multiplex proteins assay
- ✓ **Sorting**

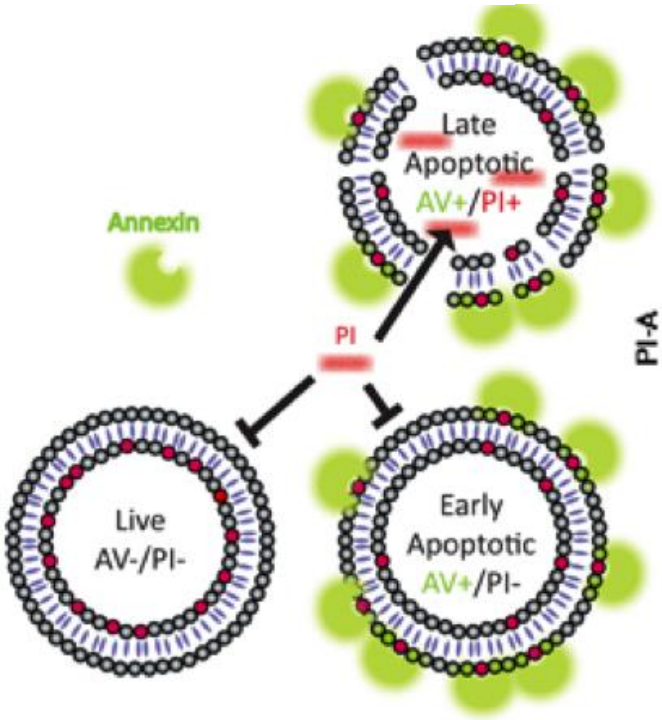
Cell cycle

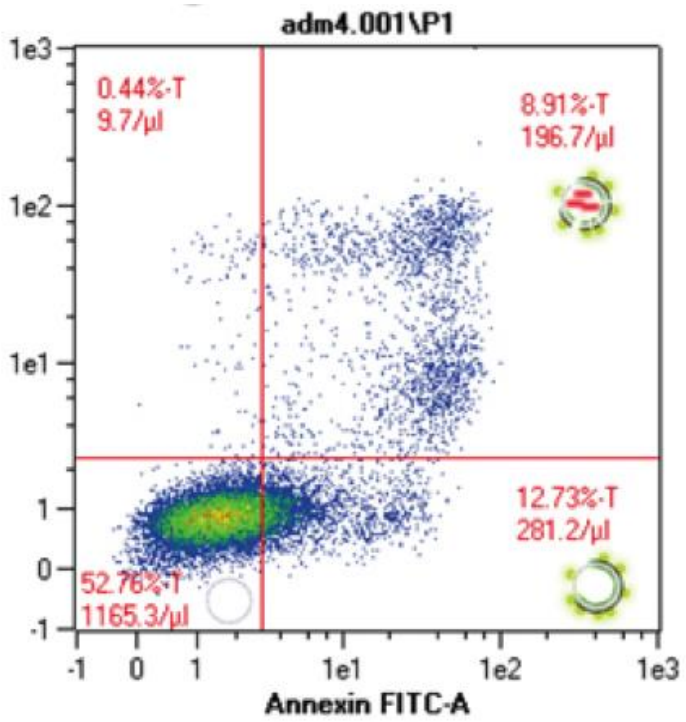

A
M
G2
G1
S
B
M
DNA content
2
1
0
G1
S
G2
Cell age
C
G1
Cell number
G2M
S
1.0
2.0
DNA content


Legend
Sub G1
G0/G1
S
G2/M
129
103
77
52
26
0
Count
0
4000
8000
12000
16000
20000
PI (intensity)

Apoptosis (Anexcine V)

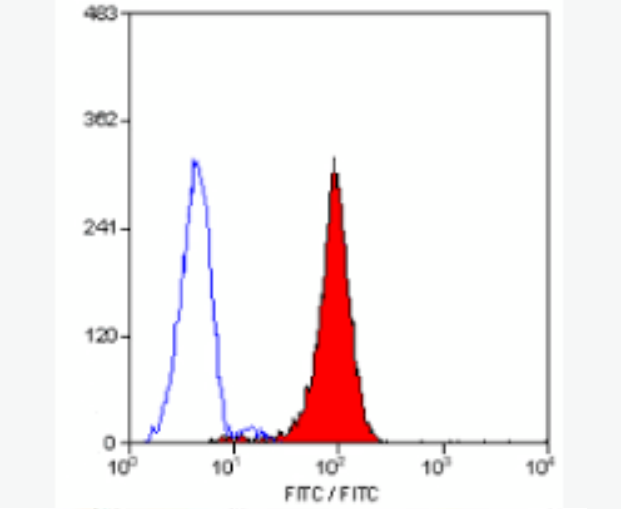




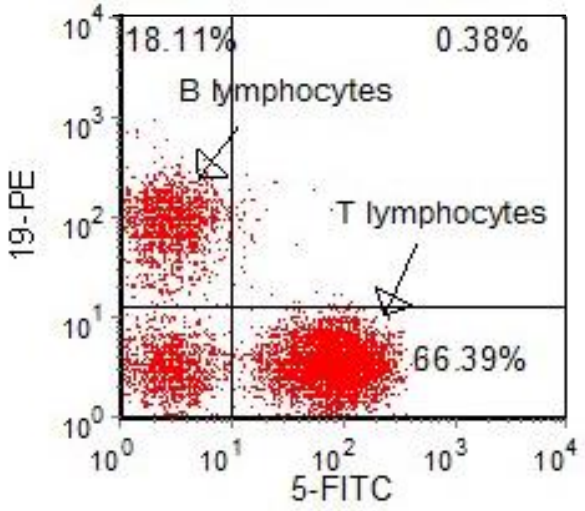


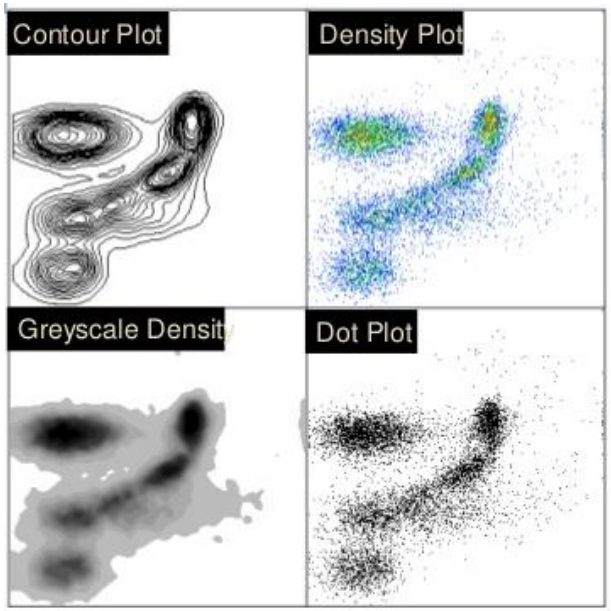
Plot Types

1 color



2 color 이상





T cell panel Antibody staining 조건

		Step 1	Step 2						Step 3	
[T cell]		FC bloc/(50uL)	CD3(iso)	CD3	CD4(iso)	CD4	CD8(iso)	CD8	7AAD (/100uL)	(uL)
1	Unstain	2.5								
2	CD3(iso)	2.5	2.5	—	—	—	—	—	5	
3	CD4(iso)/CD8(iso)	2.5	—	2.5	10	—	10	—	5	
4	CD3,CD4,CD8	2.5	—	2.5	—	10	—	10	5	

		Step 1	Step 2				
[Compensation sample]		FC bloc/(50uL)	CD3	CD4	CD8	7AAD	(uL)
1	e450 (CD3)	2.5	2.5	—	—	—	
2	PE (CD4)	2.5	—	10	—	—	
3	APC (CD8)	2.5	—	—	10	—	
4	7AAD	2.5	—	—	—	5	

T cell panel Antibody staining 조건

		Step 1	Step 2						Step 3	
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1	Unstain	2.5								
2	CD3(iso)	2.5	2.5	—	—	—	—	—	5	
3	CD4(iso)/CD8(iso)	2.5	—	2.5	10	—	10	—	5	
4	CD3,CD4,CD8	2.5	—	2.5	—	10	—	10	5	

		Step 1	Step 2				
[Compensation sample]		FC bloc/(50uL)	CD3	CD4	CD8	7AAD	(uL)
1	e450 (CD3)	2.5	2.5	—	—	—	
2	PE (CD4)	2.5	—	10	—	—	
3	APC (CD8)	2.5	—	—	10	—	
4	7AAD	2.5	—	—	—	5	

Antibody staining process

[Immune cell Surface marker stain protocol]

Cell (5*10^5~2*10^6)/tube 준비 (1*10^5~1*10^8까지 가능)

① FACS buffer 1ml로 washing – 400g, 5min cfg 후 상층액 제거

② FC blocker : 2.5ul/50uL facs buffer/sample) 준비 혼합 후 RT에서 10min incubation

③ (Antibody mixture 만들기) total 50uL /sample

④ ②번 tube에 antibody mixture 혼합 = total 100uL

⑤ 4C에서 30min incubation

⑥ FACs buffer 1ml로 2회 washing (Cfg. 400g. 5min)

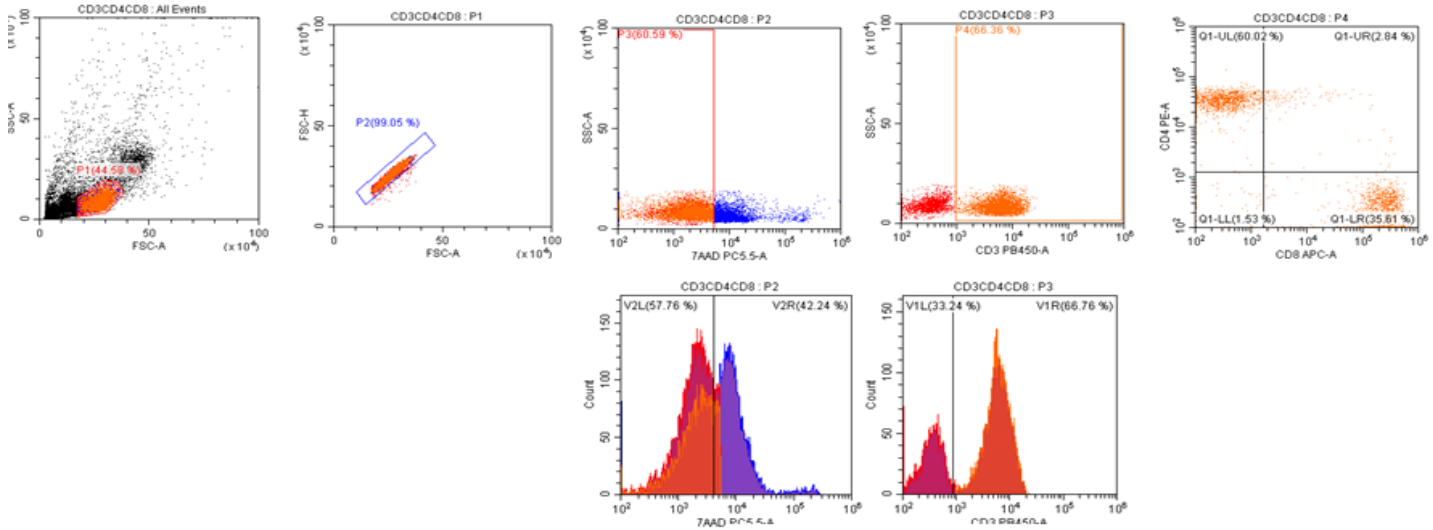
⑦ 100uL facs buffer로 cell 풀어서 FACS mash 통과

⑧ 7AAD 5ul/100ul sample 혼합 후 5분이상 RT incubation

⑨ 0.8% PFA buffer 100uL 혼합 (최종 0.4% PFA 고정) 후 FACS 분석

2020.06.20 workshop FACS analysis result : [T cell population]

- 분석 시행일 : 2020.06.20
- 분석 시행자 : 정주연
- Sample : Healthy donor PBMC
- 분석장비: Beckman culter – Cytoflex
- 분석panel : T cell population
- Result



Tube Name: CD3CD4CD8
Sample ID:

Population	Events	% Total	% Parent
All Events	22434	100.00 %	100.00 %
P2	9905	44.15 %	99.05 %
P3	6001	26.75 %	60.59 %
P4	3982	17.75 %	66.36 %
P1	10000	44.58 %	44.58 %
Q1-UR	113	0.50 %	2.84 %
Q1-UL	2390	10.65 %	60.02 %
Q1-LL	61	0.27 %	1.53 %
Q1-LR	1418	6.32 %	35.61 %
V1L	1995	8.89 %	33.24 %
V1R	4006	17.86 %	66.76 %
V2L	5721	25.50 %	57.76 %
V2R	4184	18.65 %	42.24 %

Tube Name: CD3CD4CD8
Sample ID:

Population	Events	% Total	% Parent
All Events	22434	100.00 %	100.00 %
P1	10000	44.58 %	44.58 %
P2	9905	44.15 %	99.05 %
P3	6001	26.75 %	60.59 %
P4	3982	17.75 %	66.36 %
Q1-UR	113	0.50 %	2.84 %
Q1-UL	2390	10.65 %	60.02 %
Q1-LL	61	0.27 %	1.53 %
Q1-LR	1418	6.32 %	35.61 %
V1L	1995	8.89 %	33.24 %
V1R	4006	17.86 %	66.76 %
V2L	5721	25.50 %	57.76 %
V2R	4184	18.65 %	42.24 %