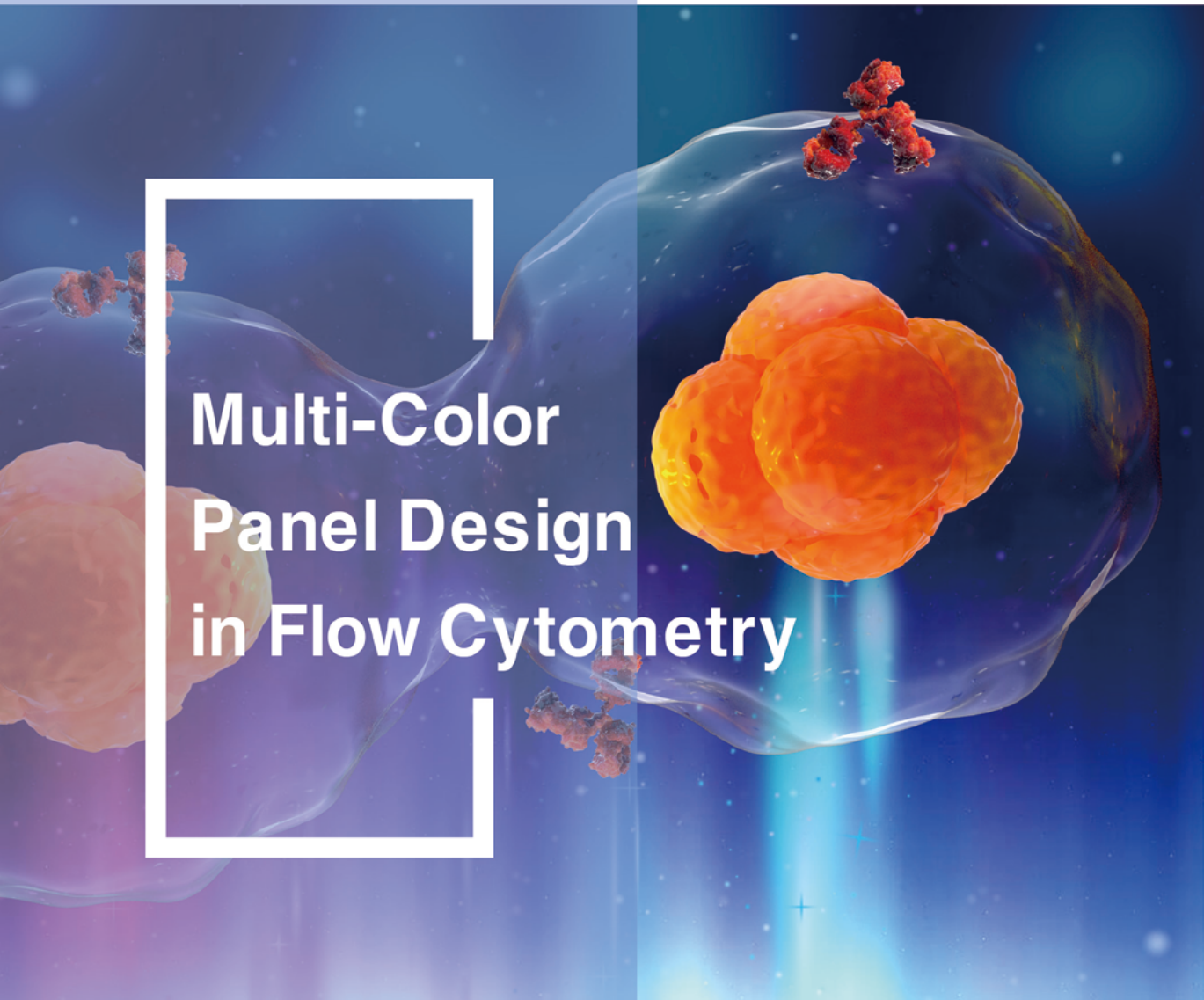


Flow Cytometry
Quick Reference Guide



Multi-Color Panel Design in Flow Cytometry

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FOCUS ON YOUR RESEARCH
SERVICE FOR LIFE SCIENCE



Elabsience

Company Introduction

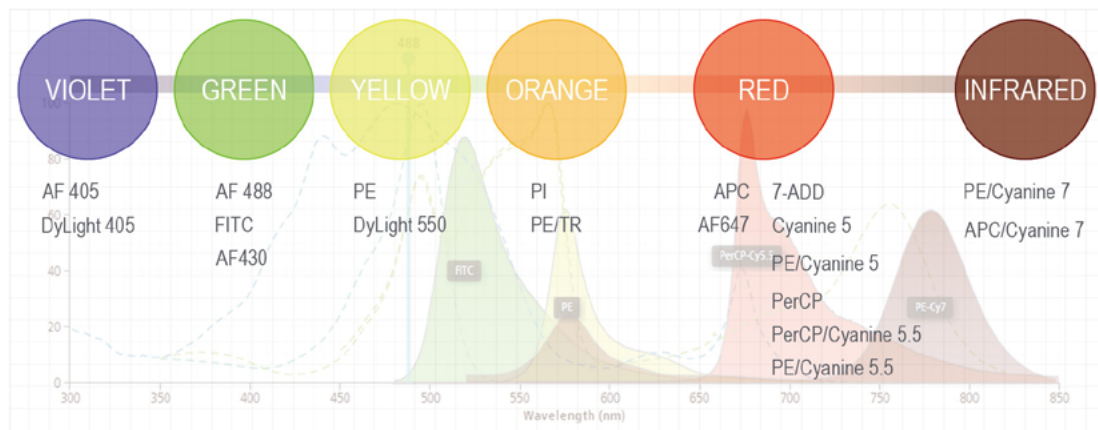
Elabsience specializes in immunodiagnostic technology for life science community. We have complete platform for R&D and manufacture. Our head office locates in the national bio-park - Wuhan Biolake in Optical Valley. We also have the US branch of innovation center in Houston, Texas.

At the same time, we have in-house QC for every product, endeavoring to keep your experiment results more consistent and precise. Elabsience products cover FCM Antibodies, Antibodies, ELISA Kits, Recombinant Proteins, Peptides, CLIA Kits, and Biochemical Kits. So far, we have 12 patents, over 5,000 citations of our products of excellent quality. Our production quality has also been certified by international quality control systems like ISO9001 and CE.

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Panel Design Principles



01 Balance Antigen Density and Fluor Brightness

High Abundance Antigen + Dim Fluorochrome

Low Abundance Antigen + Bright Fluorochrome

e.g. CD45/CD3/CD4/CD8 (Abundant Antigen) labeled with FITC (Dim Fluorochrome)



02 Avoid Spectral Overlap between Fluorochromes

Choose Fluorochrome Combination with Less Spectral Overlap to Avoid Spillover



03 Use Tandem Dyes Carefully

Tandem Dyes are Necessary in Multi-color Panel Design

Susceptible to Light Exposure or Fixation

Follow Protocol Strictly to Avoid Tandem Dye Degradation



04 Avoid Bright Light

Most fluorescent dyes (particularly tandem dyes like PE/Cyanine7) are susceptible to exposure to bright light

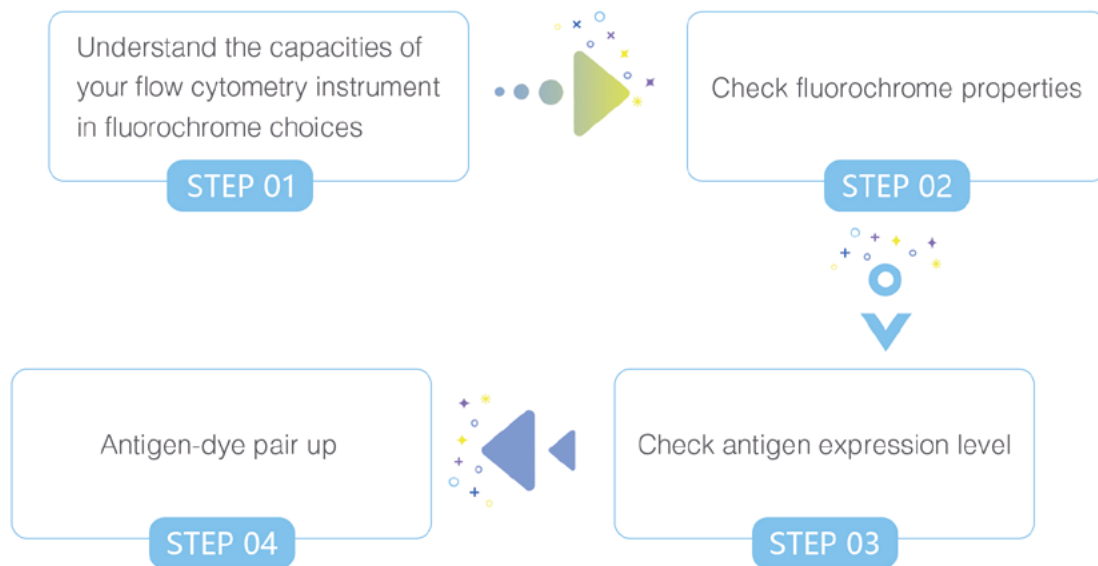


05 Cautious with Acidic Buffer and Fixation

FITC is Susceptible to Low pH Condition

Fixation and Extended Storage Could Lead to Dye Degradation

Steps in Multi-color Panel Design



STEP 01 Instrument Specification

++ Instrument Specifications in Flow Cytometry ++

Instrument	Excitation Laser Line (nm)	Channel Filter	Widely Used Fluorochromes
Flow Cytometry Instruments with 2 laser lines	488nm	530/30	FITC、AF488
		575/26	PE
		610/20	PE/TR
		695/40	PerCP/Cyanine5.5、 PE/Cyanine5、 PerCP
		780/60	PE/Cyanine7
	633nm	660/20	APC、 AF647
		730/45	AF700
		780/60	APC/Cyanine7



STEP 02 Check Fluorochrome Information

Mostly Used Fluorochrome Specifications

Fluorochrome	Emission Color	Excitation Laser Line (nm)	Em-Max (nm)
DyLight 405	Violet	405	428
AF 405	Violet	405	421
AF 488	Green	488	519
FITC	Green	488	525
AF430	Green	405	541
PE	Yellow	488/532/561	575
DyLight 550	Yellow	561	576
PI	Orange	488/532/561	620
PE/TR	Orange	488/532/561	613
7-AAD	Red	488/532/561	647
Cyanine 5	Red	633	670
APC	Red	633/635/640	660
AF647	Red	633/635/640	668
PE/Cyanine 5	Red	488/532/561	670
PerCP	Red	488/532	675
PerCP/Cyanine 5.5	Far Red	488/532	690
PE/Cyanine 7	Infrared	488/532/561	774
APC/Cyanine 7	Infrared	633/635/640	774

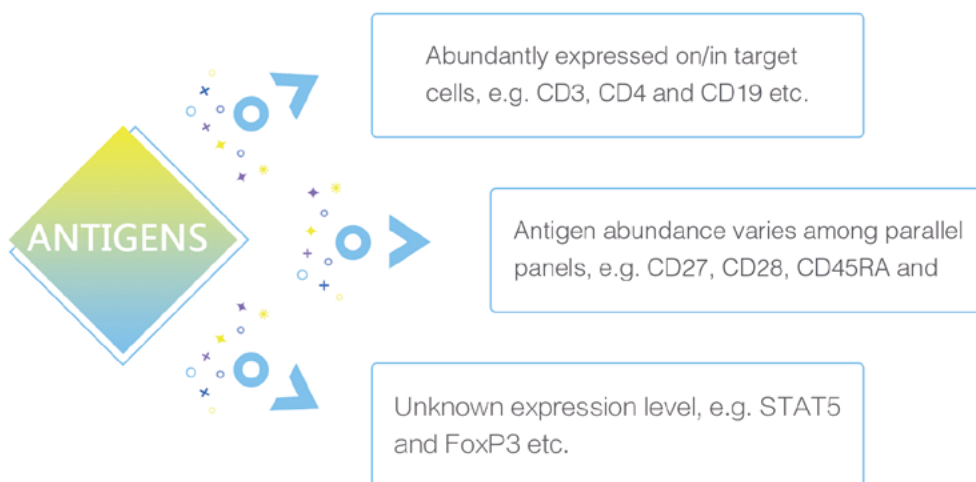
✦ Brightness of Various Fluorochromes ✦

Relative Brightness	Brightest	Bright	Medium	Dim
Fluorochrome	PE	PE/Cyanine7	AF488	PerCP
	PE/Cyanine5	AF647		
	APC	PerCP/Cyanine 5.5	FITC	APC/Cyanine 7



STEP 03 Antigen Expression Level

Based on the expression level on/in target cells, antigens could be categorized into three tiers.



++ Expression of Common Surface Molecules on Blood Cells ++

Cell Subset	Antigen	Expression Level
Lymphocytes	CD3	++
	CD4	++
	CD8	++
	CD19	+
T Cell	TCR	+++
	CD2	++
	CD3	+++
	CD5	++
	CD7	++
	CD45	++++
CD4+T Cell	CD4	+++
	CD28	++
	CCR5	++
CD8+T Cell	CD8	++
	CD28	++

"+" indicates antigen density <10,000;

"++" indicates 10,000<antigen density <100,000;

"+++" indicates 100,000<antigen density <200,000;

"++++" indicates 200,000<antigen density <300,000;

"+++++" indicates antigen density >300,000.

++ Expression of Common Surface Molecules on Blood Cells ++

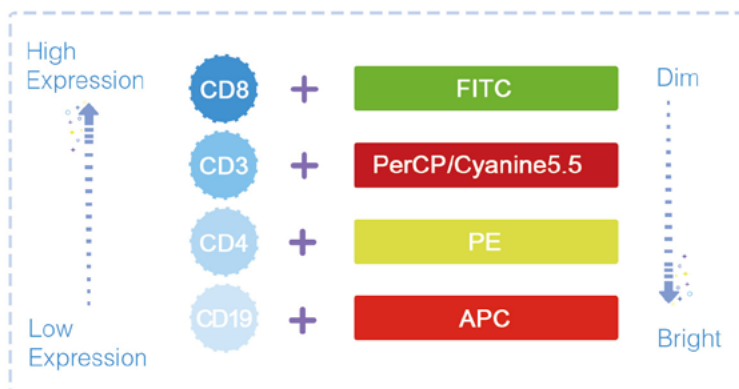
Cell Subset	Antigen	Expression Level
B Cell	CD19	++
	CD20	+++
	CD21	++++
	CD22	++
	HLA-DR	+++
	CD11a	++
	CD40	+
	CD86	++
	CD80	+
Dendritic Cell	CD11a	++
	CD40	++
	CD80	+++
	CD86	++++
Monocyte	CD14	+++
	CD32	++
	CD64	++
Neutrophil	CD14	+
	CD16	++++
NK Cell	CD56	++

- ★ "+" indicates antigen density <10,000;
- ★ "++" indicates 10,000<antigen density <100,000;
- ★ "+++ " indicates 100,000<antigen density <200,000;
- ★ "++++" Indicates 200,000<antigen density <300,000;
- ★ "+++++" indicates antigen density >300,000.

STEP 04 Antigen-fluorochrome Pair Up

Suggested 4 fluorochromes: FITC, PE, APC and PerCP/Cyanine5.5

Case study: Human lymphocyte subset analysis



The fluorochromes in this panel are conjugated to most of commercially available antibodies. Besides, there is little spectral overlap in this pane and, thus, guarantee an optimized result.

Suggested 3 fluorochromes: FITC, PE and APC

Suggested 5 fluorochromes: FITC, PE, APC, PerCP/Cyanine5.5 and PE/Cyanine7

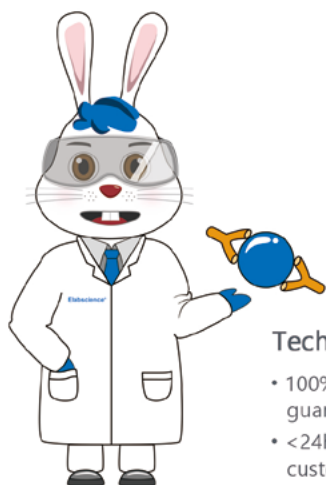
Suggested 6 color fluorochromes: FITC, PE, APC, PerCP/Cyanine5.5, APC/Cyanine7 and PE/Cyanine7

Fluorochrome	Laser Line nm	Em-Max nm	Ex-Max nm	Filter	Brightness	Properties
FITC	488	490	525	530/30	Medium	Susceptible to acidic buffer
PE	488/561	496/565	575	575/26	Bright	Bright and relatively stable
APC	633	650	660	660/20	Bright	Bright but not as stable as PE
PerCP/Cyanine5.5	488	490	690	695/40	Medium	Relatively robust against light exposure and fixation
APC/Cyanine 7	633	650	775	780/60	Dim	Susceptible to light exposure and fixation
PE/Cyanine7	488/561	496/565	775	780/60	Bright	Susceptible to light exposure and fixation

Suggested 8 color fluorochrome: FITC, PE, APC, PerCP/Cyanine5.5, APC/Cyanine7, PE/Cyanine7, AF430 and PE/TR

Elabscience®

Brings greater value to your Flow Cytometry experimnts by offering reliable product quality, technical support and competitive price.



Tech Support

- 100% Customer satisfaction guarantee
- <24h Feedback time to customer inquiry
- Multi-colour panel design



Quality

- Cover mostly cited clones
- Reliable quality
- Focus on antibody development and quality control

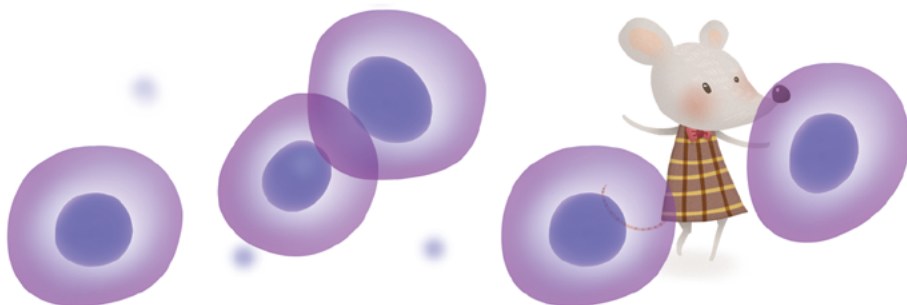
Price

- Very competitive price
- Save up to half of expense on FC antibodies for researchers

Recommended Panels for Lymphocyte Phenotyping

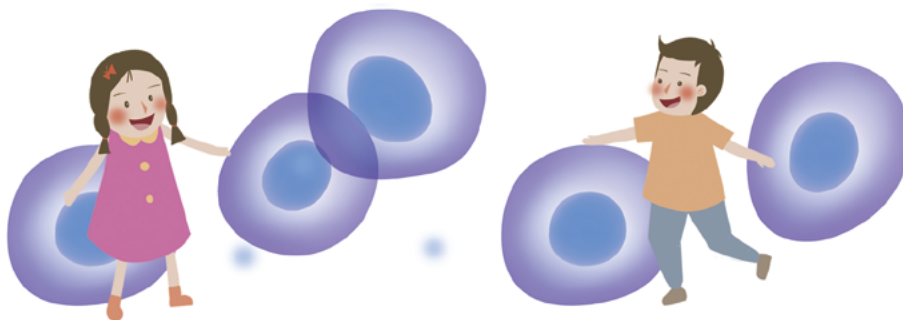
○ ○ Suggested Panels for Mouse Lymphocyte Phenotyping ○ ○

	Target Cell	Antigen	Fluorochrome	Clonity	Cat Number	Size
Mouse	T cell	CD3ε	APC	145-2C11	E-AB-F1103E	50 /100 /500 Tests
					E-AB-F1103UE	25 /50 /100 /200 µg
		CD4	PE	GK1.5	E-AB-F1097D	100 /200 /500 Tests
					E-AB-F1097UD	50 /100 /200 µg
		CD8a	FITC	53-6.7	E-AB-F1104C	100 /200 /500 Tests
					E-AB-F1104UC	25 /50 /100 /200 µg
	B cell	CD3	FITC	17A2	E-AB-F1013C	100 /200 /500 Tests
					E-AB-F1013UC	50 /100 /200 µg
		CD19	APC	1D3	E-AB-F0986E	50 /100 /500 Tests
					E-AB-F0986UE	25 /50 /100 /200 µg
	NK cell	NK1.1	PE	PK136	E-AB-F0987D	50 /100 /500 Tests
					E-AB-F0987UD	25 /50 /100 /200 µg
		CD49b	APC	DX5	E-AB-F1116E	50 /100 /200 /500 Tests
					E-AB-F1116UE	25 /50 /100 /200 µg
	Treg cell	CD4	FITC	GK1.5	E-AB-F1097C	100 /200 /500 Tests
					E-AB-F1097UC	50 /100 /200 µg
		CD25	AF647	PC-61.5.3	E-AB-F1102M	20 /50 /100 /200 Tests
					E-AB-F1102UM	25 /50 /100 /200 µg
		CD127	PE	A7R34	E-AB-F1023D	20 /50 /100 /200 Tests
					E-AB-F1023UD	25 /50 /100 /200 µg
	Treg cell	CD4	FITC	GK1.5	E-AB-F1097C	100 /200 /500 Tests
					E-AB-F1097UC	50 /100 /200 µg
		CD25	AF647	PC-61.5.3	E-AB-F1102M	20 /50 /100 /200 Tests
					E-AB-F1102UM	25 /50 /100 /200 µg
		FOXP3	PE	150D	E-AB-F1208D	20 /50 /100 /200 Tests



○ ○ Suggested Panels for Human Lymphocyte Phenotyping ○ ○

	Target Cell	Antigen	Fluorochrome	Clonity	Cat Number	Size
Human	T cell	CD3	FITC	OKT3	E-AB-F1001C	20 /50 /100 /200 Tests
		CD4	PerCP/ Cyanine5.5	RPA-T4	E-AB-F1109J	20 /50 /100 /200 Tests
		CD8a	APC	OKT-8	E-AB-F1110E	20 /50 /100 /200 Tests
	B cell	CD3	FITC	OKT3	E-AB-F1001C	20 /50 /100 /200 Tests
		CD19	APC	CB19/4G7	E-AB-F1004E	20 /50 /100 /500 Tests
					E-AB-F1127E	20 /50 /100 /200 Tests
	NK cell	CD16	APC	CB16	E-AB-F1005E	20 /50 /100 /200 Tests
		CD56	PE/Cyanine7	HCD56	E-AB-F1210H	20 /50 /100 /200 Tests
	NKT cell	CD3	FITC	OKT3	E-AB-F1001C	20 /50 /100 /200 Tests
		CD56	PE/Cyanine7	HCD56	E-AB-F1210H	20 /50 /100 /200 Tests
	Treg cell	CD4	FITC	RPA-T4	E-AB-F1109C	20 /50 /100 /200 Tests
		CD25	APC	BC96	E-AB-F1194E	20 /50 /100 /200 Tests
		CD127	PE	A019D5	E-AB-F1152D	20 /50 /100 /200 Tests
	Treg cell	CD4	FITC	RPA-T4	E-AB-F1109C	20 /50 /100 /200 Tests
		CD25	APC	BC96	E-AB-F1194E	20 /50 /100 /200 Tests
		FOXP3	PE	150D	E-AB-F1208D	20 /50 /100 /200 Tests





Elabscience Biotechnology Inc.

Web: www.elabscience.com

E-mail: techsupport@elabscience.cn

Tel: 1-240-252-7368

Fax: 1-240-252-7376

Add: 14780 Memorial Drive, Suite 216, Houston, Texas 77079.