

Cytoflex 2

Flow Cytometry & Single Cell Analysis https://fcsc.ku.dk/

										Se	condary Flo	urochrom	e (Bright	Antigen)									
	BV421	Pac Blue	BV480	BV510	BV605	BV650	FITC	BB515	AF 488	PerCP-Cy	5.5 BB700) PE	F	PE-CF594 PE	-Cy5	PE-Cy7	APC	AF 647	AF 700	APC-R700	APC-Cy7	APC-Fire	APC-eflour780
BV421	683		59%	6 19%	39%	30%	:	1%	0% 0)%	0%	1%	0%	2%	3%	1%		0%	5%	5% 1%	6 29	6%	29
Pacific Blue		69	59%	<mark>6</mark> 20%	39%	30%	:	1%	0% 0)%	0%	1%	0%	2%	3%	1%		0%	5%	5% 1%	6 29	6%	29
BV480	16%	2%	208	3	0%	6 0%	!	5%	0%)%	0%	0%	0%	0%	0%	0%		0%	0%	7% 0%	6 29	6 0%	19
BV510	16%			96	_			5%	0%)%	0%	0%	0%	0%	0%	0%				7% 0%	6 29	6 0%	19
BV605	4%	17%	50%	61%	347	62%		1%	0% 12	!%	9%	30%	80%	89%	50%	0%	2	3% 1	2%	5% 3%	6 19	6 11%	5 09
BV650	3%	0%	33%	6 42%	74%	146		1%	0% 0)%	21%	59%	34%	48%	81%	0%		2%	0%	1% 5%	6 19	6 0%	
FITC	0%	5%	66%	6 0%	0%	6 0%	1	32			10%	25%	24%	12%	23%	20%	1	6% 2	9% 3	0% 29%	6 309	6 31%	
BB515	0%	5%	66%	6 0%	0%	6 0%			552		10%	25%	24%	12%	23%	20%	1	6% 2	9% 3	0% 29%	6 309	6 31%	
Alexa Fluor 488	0%	5%	66%	6 0%	0%	6 0%			1	84	10%	25%	24%	12%	23%	20%	1	6% 2	9% 3	0% 29%	6 309	6 31%	
PerCP-Cy5.5	0%	0%	0%	6 0%	819	77%	4	<mark>4%</mark> :	11% 3	3%	92		82%	93%	98%	25%	9	5% 8	<mark>7%</mark> 6	3% 95%	539	<mark>6</mark> 1%	
BB700	0%	0%	0%	6 0%	81%	77%	4	<mark>4%</mark> 1	11% 4	1%		850	82%	93%	98%	25%	9	5% 8	<mark>7%</mark> 6	3% 95%	539	<mark>6</mark> 1%	179
PE	0%	0%	4%	6 5%	879	13%	. :	1%	1% 5	5%	5%	2%	1277	83%	46%	21%		7%	6%	5% 11%	6 59	6 7%	5 59
PE-CF594	1%	5%	15%	25%	949	42%	:	1%	0% 3	3%	3%	7%	83%	925	20%	6%		6%	4%	1% 19	6 2 9	6 5%	19
PE-Cy5	0%	0%	0%	6 0%	72%	83%	30	5%	0% 0)%	88%	92%	73%	86%	1611	14%	9	5% 8	<mark>8%</mark> 7	96%	569	<mark>6</mark> 3%	
PE-Cy7	0%	0%	0%	6 0%	48%	26%	(0%	0% 1	.%	54%	77%	48%	76%	94%	1174	4	8% 4	<mark>1%</mark> 3	4% 86%	6 929		_
APC	0%	0%	5%	6%			!	5%	0%)%	42%	76%	18%	38%	98%	14%	3	66	2	9% 75%	539	<mark>6</mark> 21%	
Alexa Fluor 647	0%	0%	5%	6%	36%	83%	!	5%	0% 0)%	42%	76%	18%	38%	98%	14%				75%			
Alexa Fluor 700	0%	8%	0%			59%		7%	1%	9%	70%	97%	47%	79%	95%	4%	8	2 % 8	3%	66	339	6 23%	
APC-R700	0%	8%	0%	6%	43%	59%		7%	1% 9)%	70%	97%	47%	79%	95%	4%	8	2% 8	3%	493	339	6 23%	259
APC-Cy7	0%	1%	0%	6 0%			:	1%	0%		-	75%	2%	14%	80%	93%	7	8% 7	4% 6	1% 89%	23:	_	
APC-Fire	0%	1%	0%	6 0%	2%	41%	:	1%	0%)%	42%	75%	2%	14%	80%	93%	7	8% 7	4% 6	1% 89%	6	221	
APC-eflour780	0%	1%	0%	6 0%	29	41%		1%	0%)%	42%	75%	2%	14%	80%	93%	7	8% 7	4% 6	1% 89%	6		24

How the matrix was made

Mouse spleens was stained with individual anti-CD8 labeled antibodies with the indicated flourochrome and analyzed on the indicated instrument.

Each calculated value was arbitrarily assigned a color code according to the legend to show where the biggest spreading was situated.

How to use the resolution impact matrix

You find the color of interest on the top of the matrix, go down till you find the channel that you need to combine the color with and read the impact of spreading.

Consider this for panel design

When you are designing larger panels the task of making correct combinations becomes more difficult, but using the list below can help you:

- The lineage markers such as CD4, CD19 etc. should be found on the top of the matrix.
- Make sure the lineage marker has as many green cells as possible.
- For an important marker you should find the color on the left of the matrix.
- Make sure the marker has as many green cells as possible when you move across the matrix.
- Notice that spreading occurs between different laser lines.

Amount of spreading (%)

0-20
20-40
40-60
60-80
80-100
NA

Relative fluorochrome brightness (AU)

#