CPD Evidence 3 (2022)

1. Registrant Information

1.1 Full Name: Chen Liu, AMRSB
1.2 Profession: BMS
1.3 Registration Number: BS075665
1.4 CPD type: Formal and educational – Research
1.5 Date of completion: 29/04/2022
1.6 Standard(s) met:
Standard 2 – A registrant must identify their CPD activities are a mixture of learning activities relevant to current or future practice.

2. Details

Title: Anti-pSHP-1 antibody titration.

Commercial antibody used for flow cytometry comes at a standard concentration (unknown) which may require dilution to fit the local lab. In this project, I titrated the mouse pSHP-1 (pY564) antibody from *Invitrogen*, and run the samples on flow cytometry. Based on the results, I determined a suitable concentration is 1:2.5 to 1:3 (stock solution : FACS buffer).

Sample preparation
Spleen is harvested, from B16OVA mice, after 16 days of tumour implantation. Skin and . Isolation splenocytes were completed by mechanical (strainers) method. Single cell suspension was diluted with FACS buffer rather than PBS or culture medium to prevent any further phosphorylation. One group of cells were treated with pervanadate, a universal inhibitor of phosphatase. In this experiment, it inhibited the dephosphorylation of SHP-1, hence allowing the observation of pSHP-1.

Flow cytometry and anti-pSHP-1 titration

Antibody cocktail:

Fluorescence	Antibody
BV510-A	Mortality
BV605	CD4
BV650	CD8
PE	pSHP-1
Alexa Fluor 700	CD45
BUV395	TCRβ

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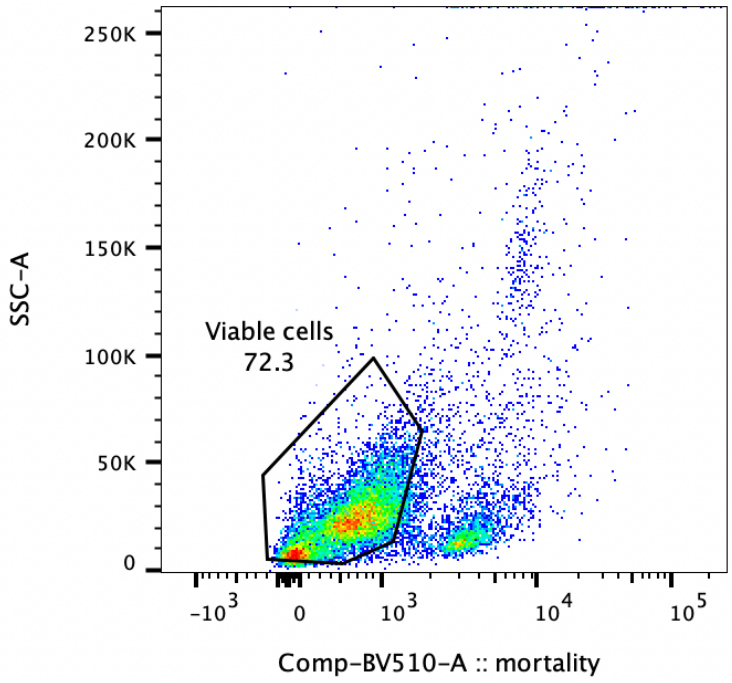
Groups:

Group	pSHP-1 concentration (/50µL)
Pervanadate non-treated	0 µL (Negative control)
	1 µL
	2.5 µL
	5 µL
	7.5 µL
Pervanadate treated	0 µL (Positive control)
	1 µL
	2.5 µL
	5 µL
	7.5 µL
FMO	CD45 FMO, no pSHP-1 Ab
	TCRβ FMO, no pSHP-1 Ab

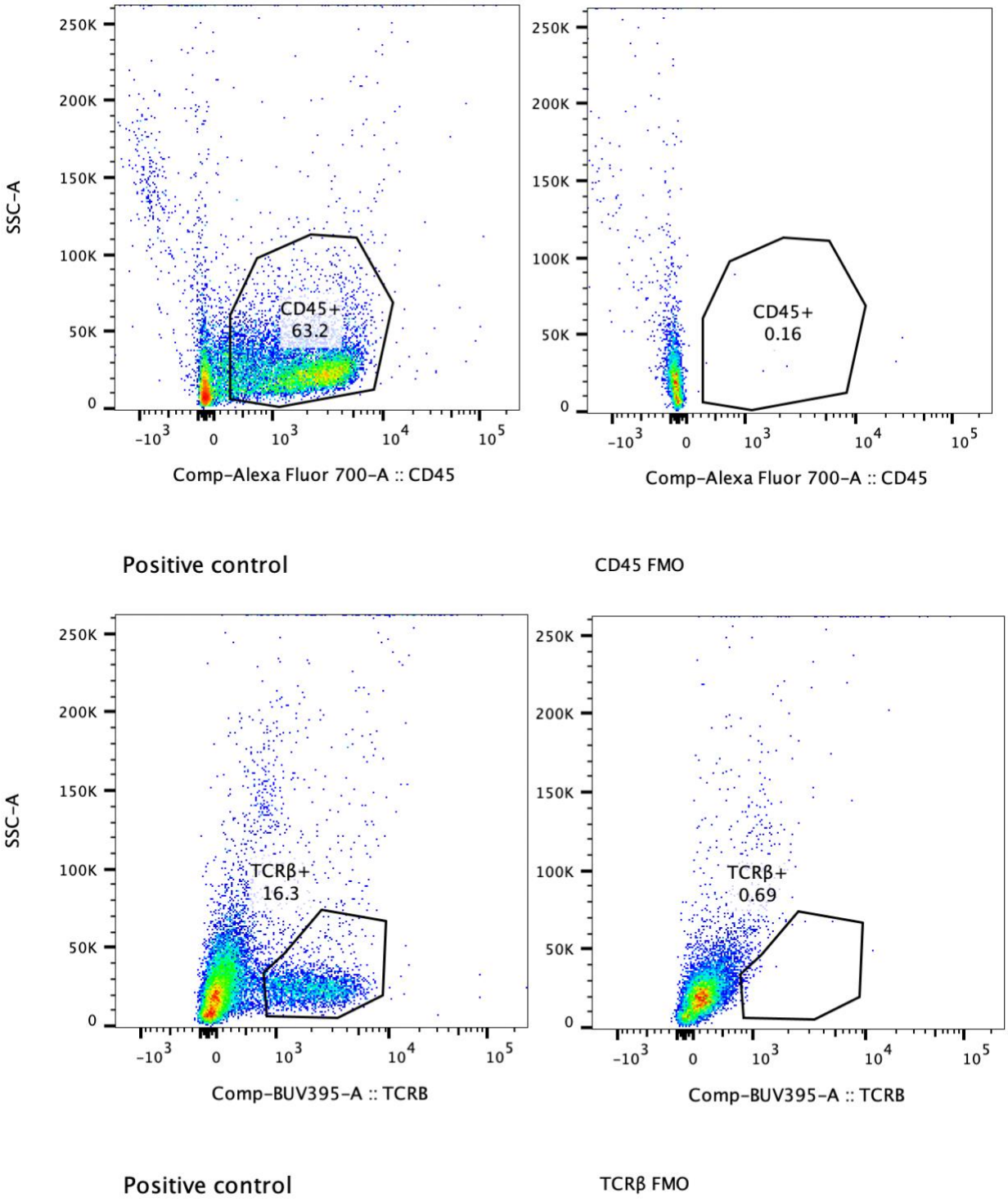
Results – Viability



Positive control

~70% of cells were viable.

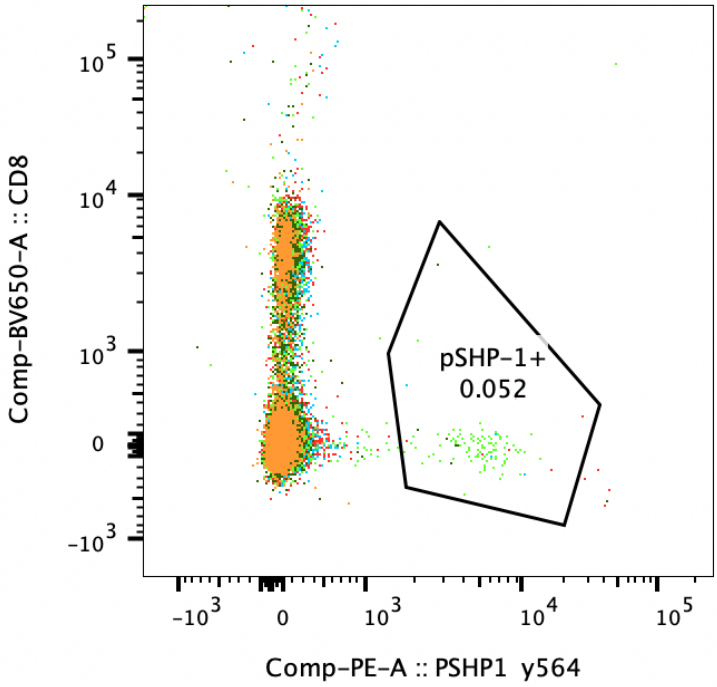
Results – FMO



Both FMO controls indicated successful antibody conjugation.

Results – pSHP-1 (pY564) titration

Based on the population of viable cells, pSHP-1 positive cells were selected:



	Sample Name	Subset Name	Count
	UT0.fcs	Viable cells	10671
	UT1.fcs	Viable cells	13527
	UT25.fcs	Viable cells	14212
	UT5.fcs	Viable cells	12436
	UT75.fcs	Viable cells	11521

For untreated group, the average percentile of pSHP-1 positive for all concentration was 0.052%.

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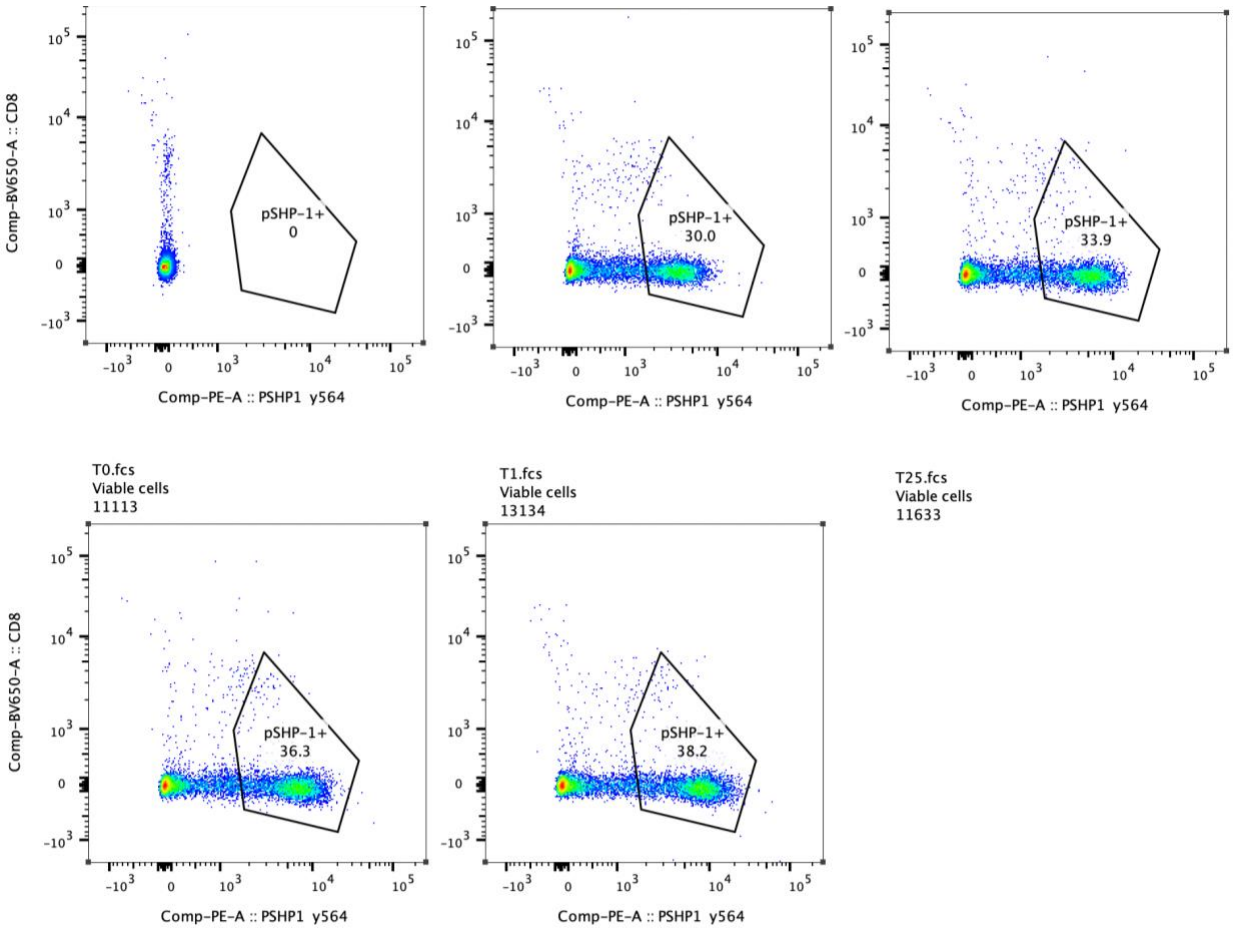

For treated group, pSHP-1 can be detected due to the inhibition of phosphatase by pervanadate.

As there were no significant difference between higher (5, 7.5µL/50µL) and lower (1, 2.5 µL/ 50µL, 2, 5:100), the suitable concentration can be elucidated as 1:2.5 to 1:3.