

anti-human CD19 (clone EPR5906) with pig PBMCs

USDA-ARS-NADC

Conclusion: the anti-human CD19 monoclonal antibody, EPR5906, does not specifically label porcine B cells

Test #1 – Extracellular titration of CD19

Maybe something at highest concentration or could just be non-specific binding of secondary antibody

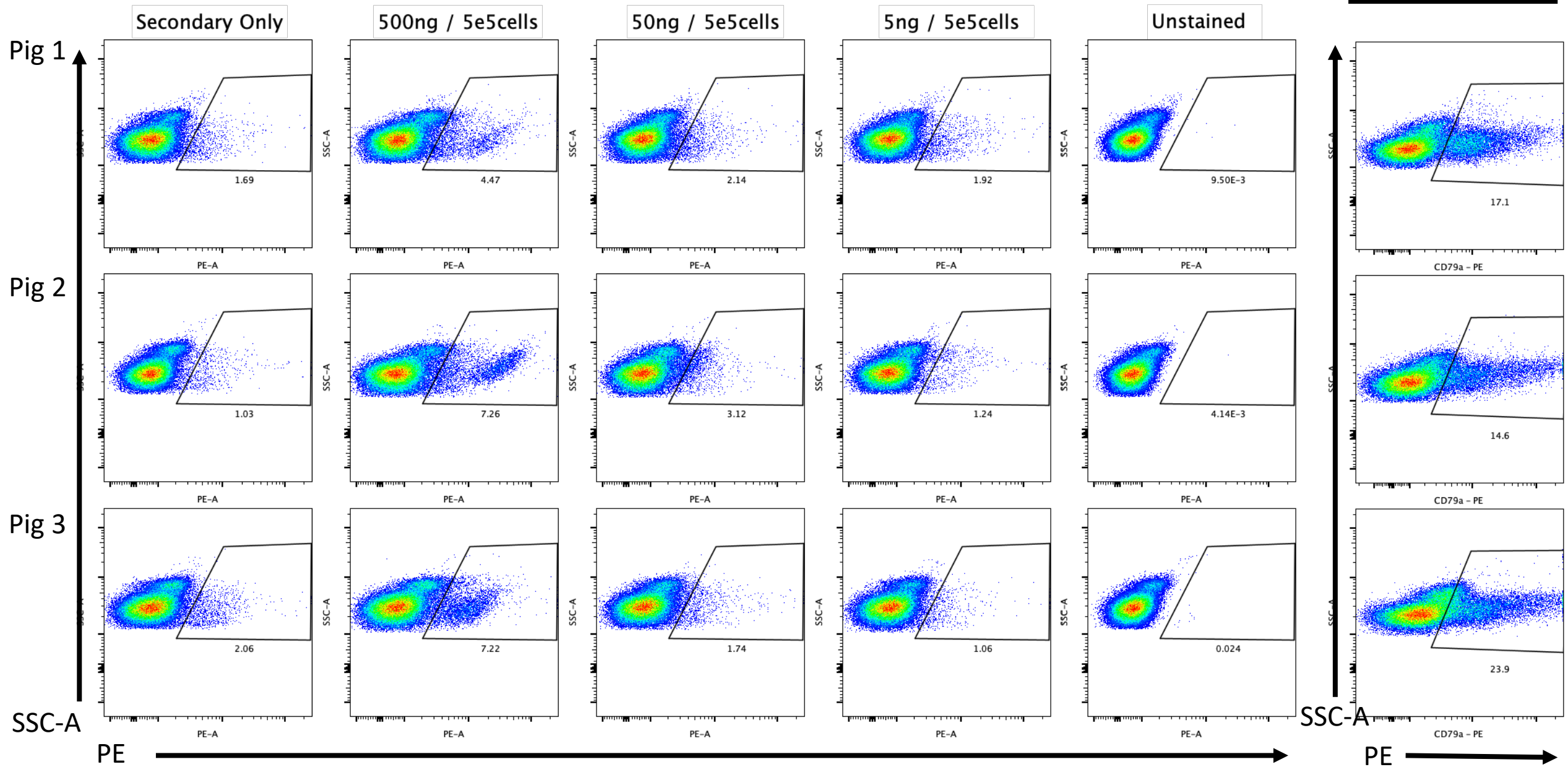
Staining order	Marker	Clone	Isotype	Channel	Concentration	Dilution	Company	Cat#	ug/rxn
1	huCD19	EPR5906	rbIgG	--	1.223mg/ml	12ul/ml	abcam	134114	0.5ug
2	rabbit Ig	SB87a	rtIgG2a	PE	0.1mg/ml	20ul/ml	Southern	4065-09	0.1ug

Basic flow panel – started with 5e5 fresh PBMCs from 3 different pigs then did serial 1:10 dilutions to give titration range of 1000ng, 500ng, 50ng, and 5ng per 5e5 PBMCs.

All primary stains were followed with 2 washes and staining with 0.2ug of anti-rabbit secondary (PE channel)

Extracellular CD19 staining of porcine PBMCs

Intracellular CD79a



Note: CD19 and CD79a staining was on the same cell samples, but NOT in the same wells

Test #1 – Intracellular titration of CD19

Nothing looks promising. But no positive control to show that the staining “works”.

Staining order	Marker	Clone	Isotype	Channel	Concentration	Dilution	Company	Cat#	ug/rxn
1	huCD19	EPR5906	rbIgG	--	1.223mg/ml	12ul/ml	abcam	134114	0.5ug
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Intracellular staining of porcine PBMCs (5e5 cells/well) with anti-human recombinant CD19

No Stain

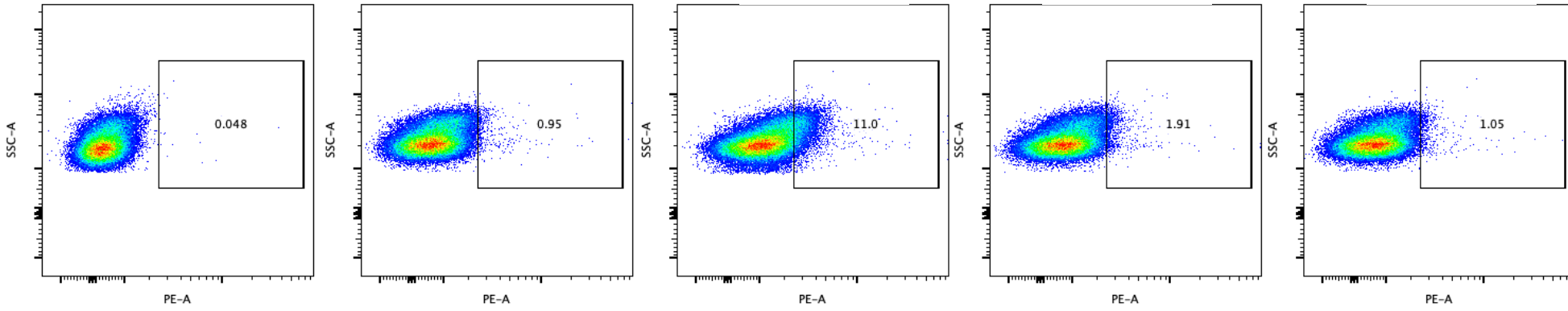
Secondary

1000ng

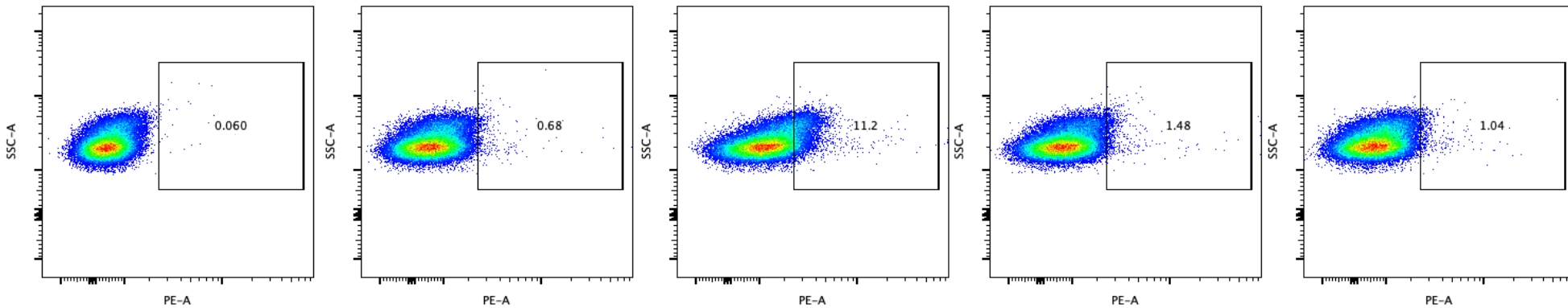
500ng

50ng

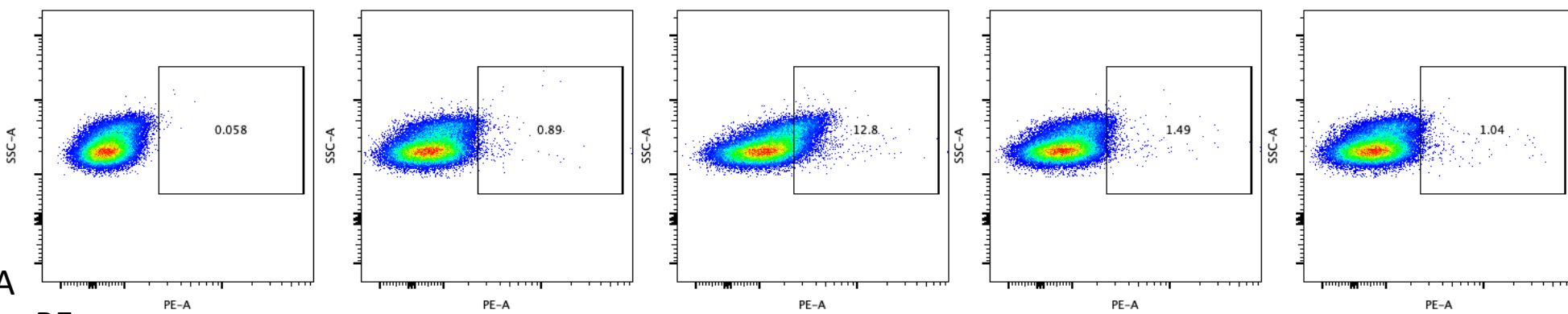
Pig 1



Pig 2



Pig 3



SSC-A

PE

Test #3 – Intracellular and extracellular with CD19 on pig PBMCs with human PBMC control
 Any staining of pig PBMCs in the CD19 channel(s) is not believable. Looks like non-specific binding of secondary.
 Extracellular CD19 then intracellular CD79a didn't work, not sure why not.

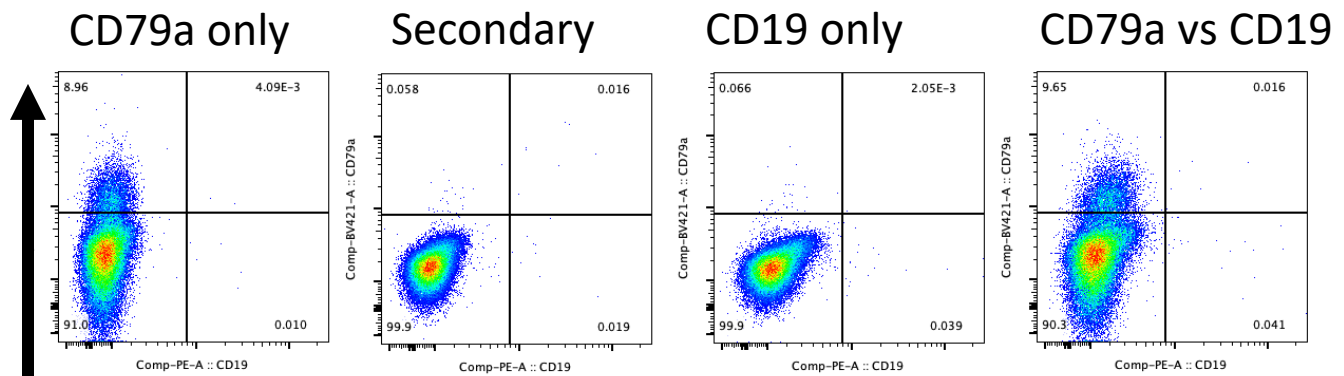
Staining order	Marker	Clone	Isotype	Channel	Concentration	Dilution	Company	Cat#	ug/rxn
1	huCD19	EPR5906	rbIgG	--	1.223mg/ml	12ul/ml	abcam	134114	0.5ug
2 panel1	rabbit IgG	polyclonal	gtIgG	AF488	2 mg/ml	1ul/ml	abcam	150077	0.2ug
2 panel2	rabbit Ig	SB87a	rtIgG2a	PE	0.1mg/ml	20ul/ml	Southern	4065-09	0.1ug
3	CD79a	HM47	msIgG1	BV421	5ul / test	50ul/ml	BD	562852	0.06ug

Basic flow panel – started with ~5e5 fresh PBMCs from 3 different pigs and 1 fresh human PBMC sample. CD19 and different secondaries (polyclonal goat AF488 or monoclonal rat PE) were used following our typical extracellular protocol and then fixed for intracellular staining – OR – were immediately fixed for intracellular staining and then CD19 and secondary staining. CD79a staining was always done with intracellular.

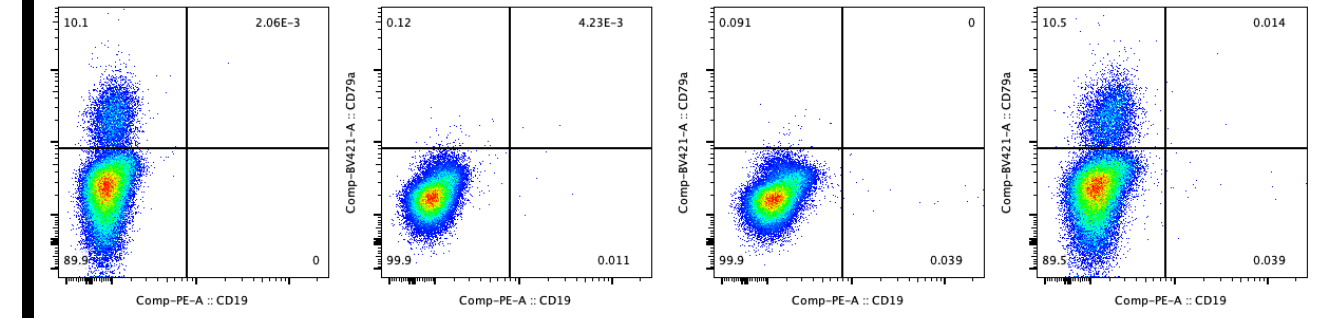
Intracellular anti-human **CD19** primary
 Intracellular anti-rabbit **PE** secondary (monoclonal rat)
 Intracellular anti-human **CD79a** **BV421** direct conjugate

- Intracellular CD79a stained both human and pig cells
- Intracellular CD19 only bound with human cells
- Human monocytes appeared to non-specifically bind the secondary a little bit

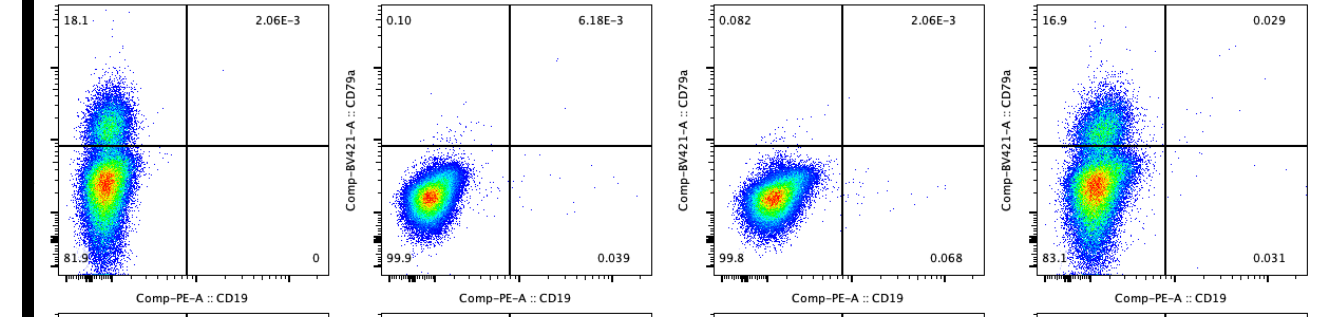
Pig 1



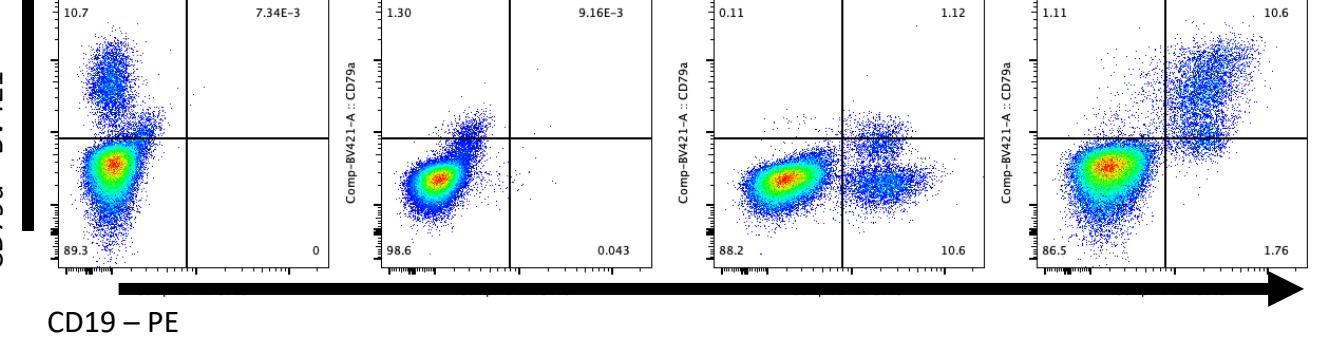
Pig 2



Pig 3



Human



CD79a - BV421

CD19 - PE

Extracellular anti-human **CD19** primary
 Extracellular anti-rabbit **PE** secondary (monoclonal rat)
 Intracellular anti-human **CD79a** **BV421** direct conjugate

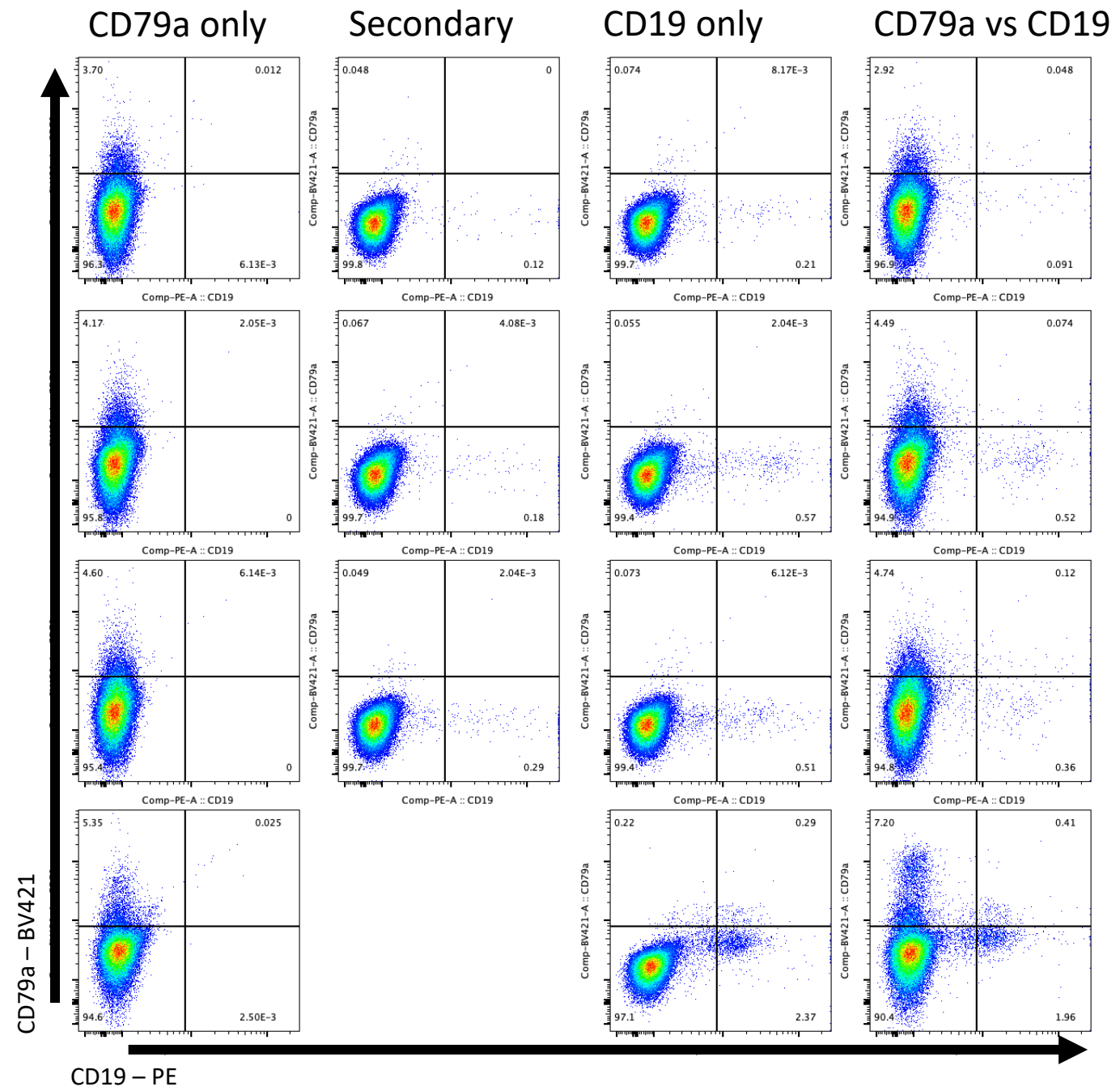
- Extracellular CD19 didn't bind human or pig PBMCs
- Secondary had some non-specific binding with monocytes again
- CD79a although intracellular stained, did not stain as well – not sure why.

Pig 1

Pig 2

Pig 3

Human



Intracellular anti-human **CD19** primary
 Intracellular anti-rabbit **AF488** secondary (polyclonal goat)
 Intracellular anti-human **CD79a** **BV421** direct conjugate

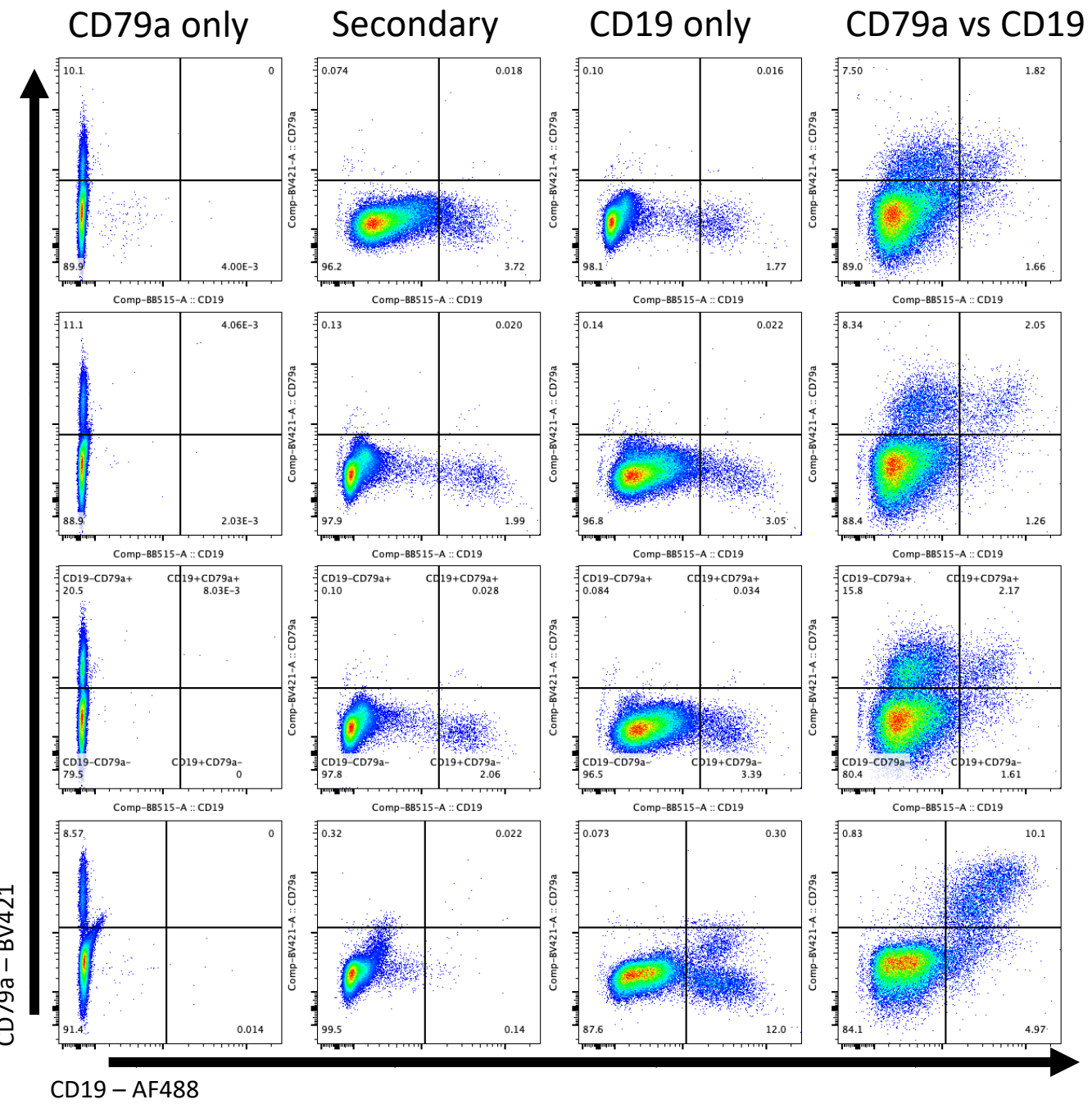
- Intracellular CD79a stained both human and pig cells
- Intracellular CD19 only bound with human cells to possible positive is just non-specific binding of the secondary

Pig 1

Pig 2

Pig 3

Human



Extracellular anti-human CD19 primary
 Extracellular anti-rabbit AF488 secondary (polyclonal goat)
 Intracellular anti-human CD79a BV421 direct conjugate

- Extracellular CD19 didn't bind human or pig PBMCs
- Secondary had some a good amount of non-specific binding to cells
- CD79a although intracellular stained, did not stain as well – not sure why.

Pig 1

Pig 2

Pig 3

Human

