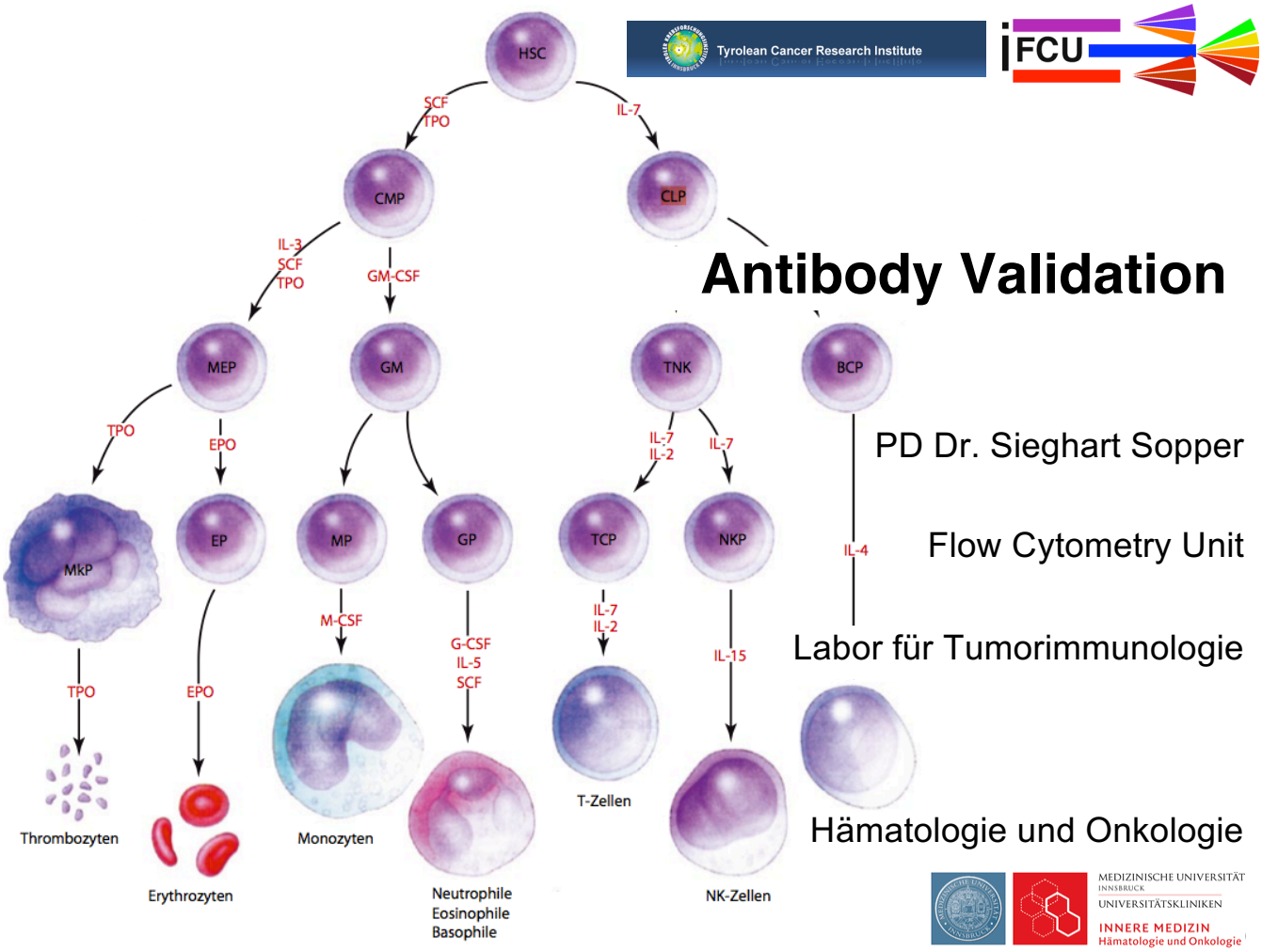


Antibody Validation

PD Dr. Sieghart Sopper

Flow Cytometry Unit

Labor für Tumorimmunologie

Hämatologie und Onkologie

Antibodies: structure

1200 AS, 150kd

Heavy chain (D, M, G, A, E)

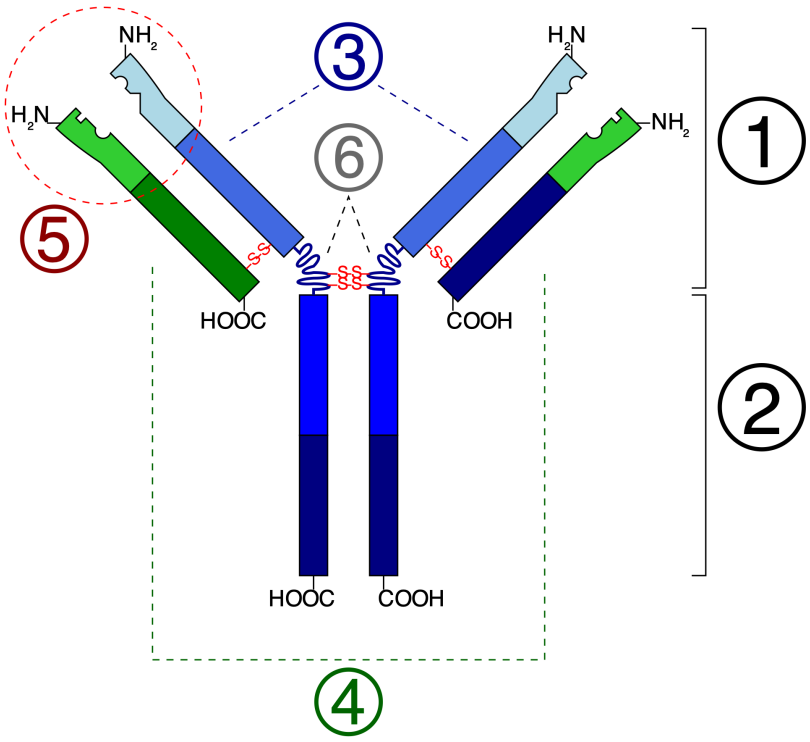
Classes

Isotype (subclasses)

Light chain (λ, κ)

Antigen binding

Constant (effector function)



Y_tambe commons.wikimedia.org

Antibodies: structure

1200 AS, 150kd

Heavy chain (D, M, G, A, E)

Classes

Isotype (subclasses)

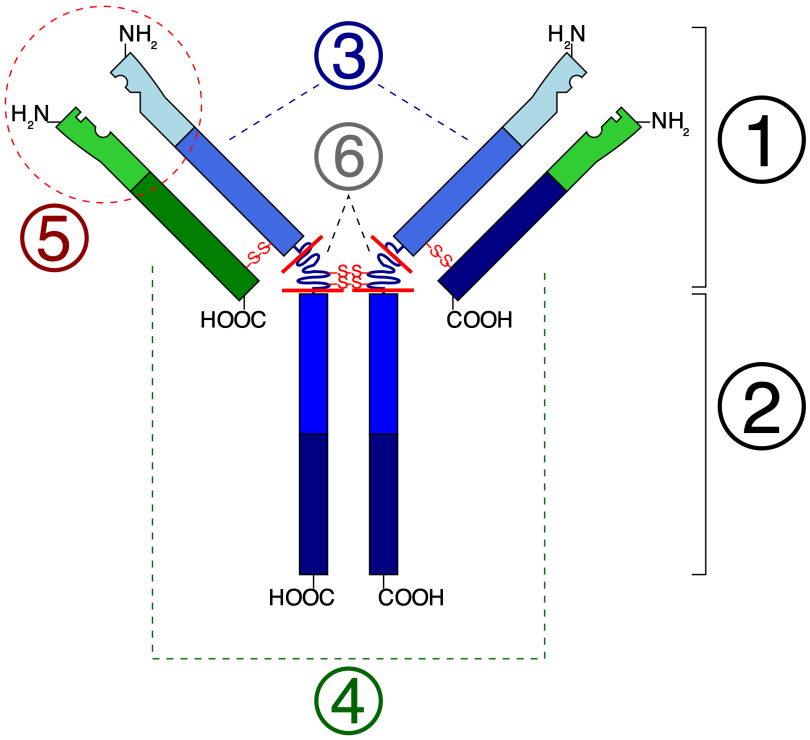
Light chain (λ, κ)

Antigen binding

Constant (effector function)

Pepsin: 2x Fab, Fc

Papain: Fab^2, 2x HC fragments



Y_tambe commons.wikimedia.org

Antibodies: types

1200 AS, 150kd

Heavy chain (D, M, G, A, E)

Classes

Isotype (subclasses)

Light chain (λ, κ)

Antigen binding

Constant (effector function)

Pepsin: 2x Fab, Fc

Papain: Fab^2, 2x HC fragments

Species

Polyclonal (Immunogen)

Monoclonal (clone)

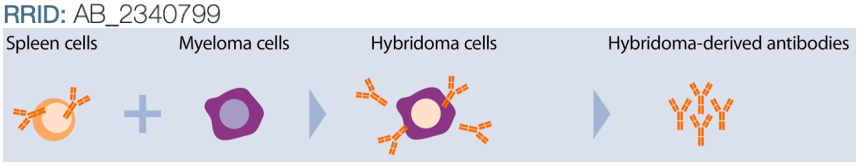
Recombinant

R-Phycoerythrin AffiniPure $F(ab')_2$ Fragment Donkey Anti-Mouse IgG (H+L)

(min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, **Rat**, Shp Sr Prot)

Conjugate Options

Target: Mouse
Host: Donkey
Antibody Format: $F(ab')_2$ Fragment
Specificity: IgG (H+L)
Minimal Cross Reactivity: Bovine, Chicken, Goat, Guinea Pig, Syrian Hamster, Horse, Human, Rabbit, Rat, Sheep Serum Proteins
Conjugate: R-Phycoerythrin
Product Category: **Fluorescent Protein Conjugates (R-PE, APC, and PerCP)**
Clonality: Polyclonal
RRID: AB_2340799



Antibodies: Affinity

$$K_A = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

K_A 10^5-10^{12}

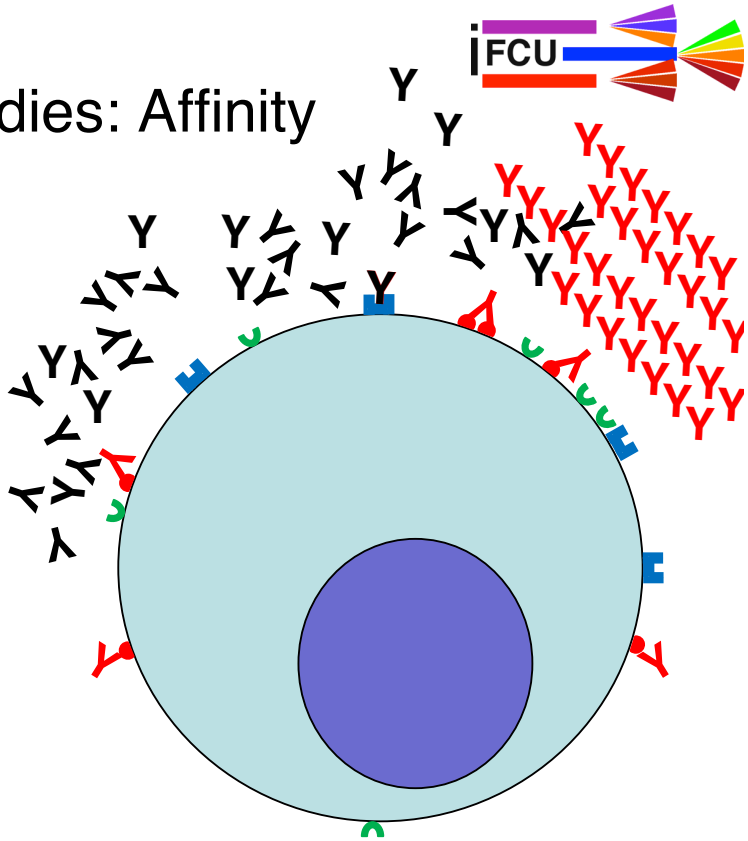
Avidin binds Biotin K_A 10^{15}

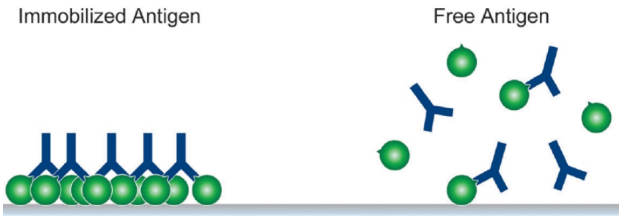
Fc receptors K_A 10^4-10^7

0,2 mg/ml Serum 18mg/ml IgG

$10^{13\text{-}15}$ AK Moleküle in 100μl

1Mio Zellen max 10^{10} Antigenmoleküle





Y_tambe commons.wikimedia.org SSOPP 0120

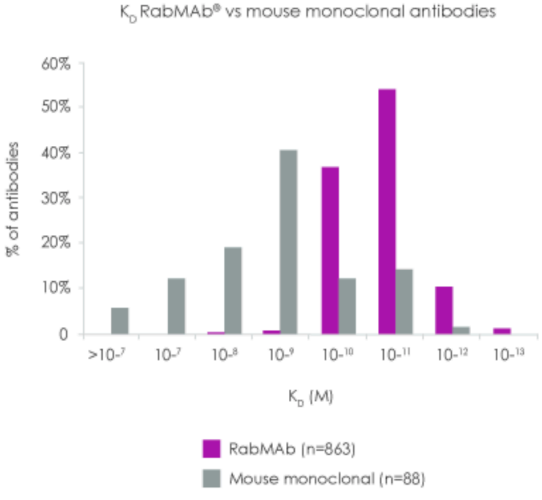
Antibodies: Affinity

$$K_A = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

Polyclonal (heterogeneous)

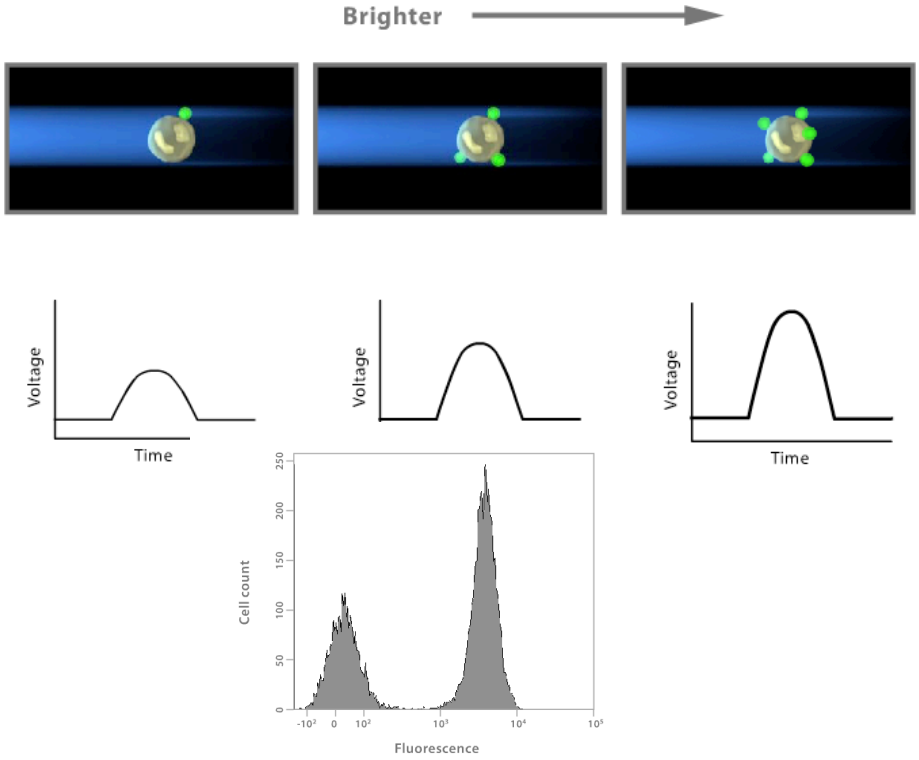
Monoclonal (defined)

Recombinant (changeable)



Advantages of recombinant antibody technology	
High affinity	Recombinant antibodies (rAbs) can be engineered to have affinities in the picomolar to femtomolar range.
High specificity	rAbs can be engineered to differentiate between similar targets.
Variety of targets	rAb targets include tissue samples, whole cells, small molecules, specific protein conformations, integral membrane proteins, RNA, post-translational modifications, and complexes, such as biotinylated major histocompatibility complex/peptide complexes.
Independent of an immune response	rAbs can be generated against unstable, toxic, volatile, immunosuppressive, or non-immunogenic antigens.
Reduced immunogenicity	Therapeutic human-derived rAbs elicit a reduced immune response compared to animal-derived antibodies.
Versatility	Generation of complete antibodies or smaller antibody fragments, like monovalent or bivalent Fab, and isotype switching using simple cloning techniques are possible.
Faster production	Automated high-throughput production; once an antibody library is established, it takes approximately 2–8 weeks to produce an rAb, in contrast to the 4+ months in animals.
Dependability	Unlike hybridoma cell lines, recombinant antibody supplies are not at risk of dying off.
Higher reproducibility	Since rAb sequences are known, they are more reliable and provide more reproducible results than mAbs.
Low lot-to-lot variability	Standardized rAb production results in low variability between different antibody lots.
Animal-free production	No animals are used unless immune antibody libraries are derived from animals.

Fluorescence



The emitted light is proportional to the number of molecules

From: http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html

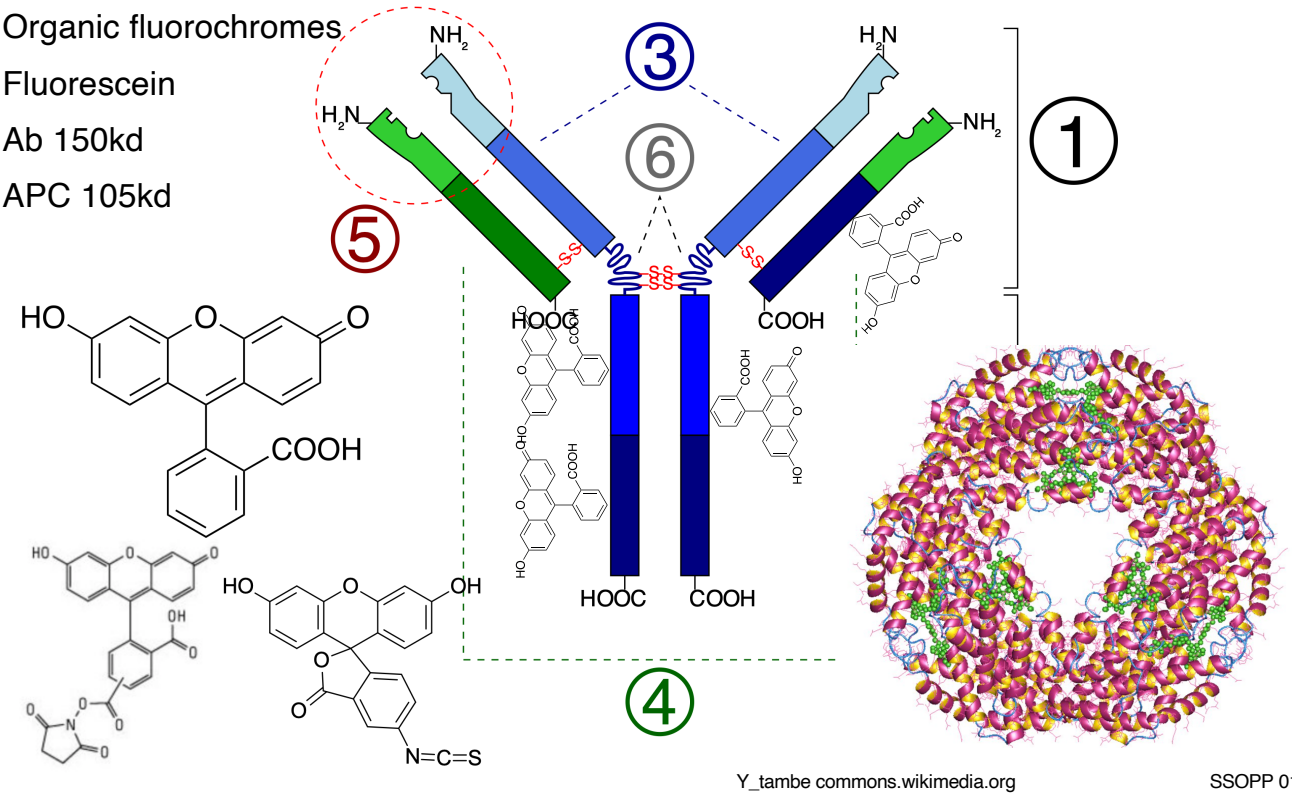
Antibodies: labelling

Organic fluorochromes

Fluorescein

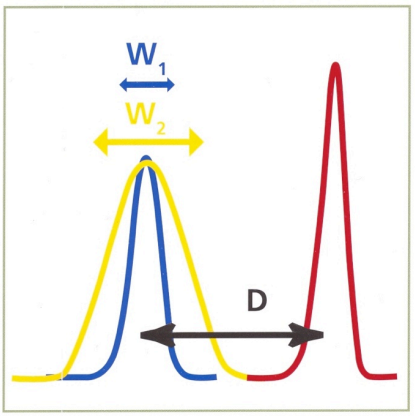
Ab 150kd

APC 105kd



Y_tambe commons.wikimedia.org

Stain index

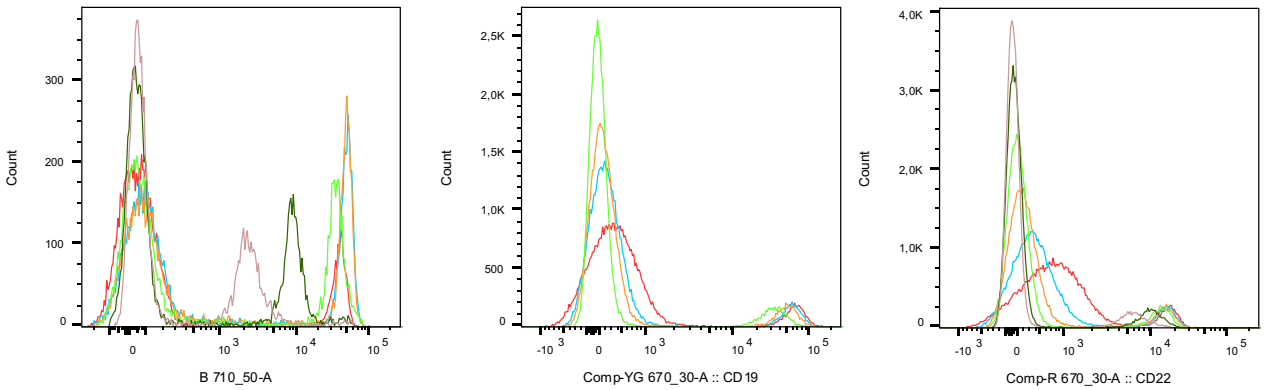


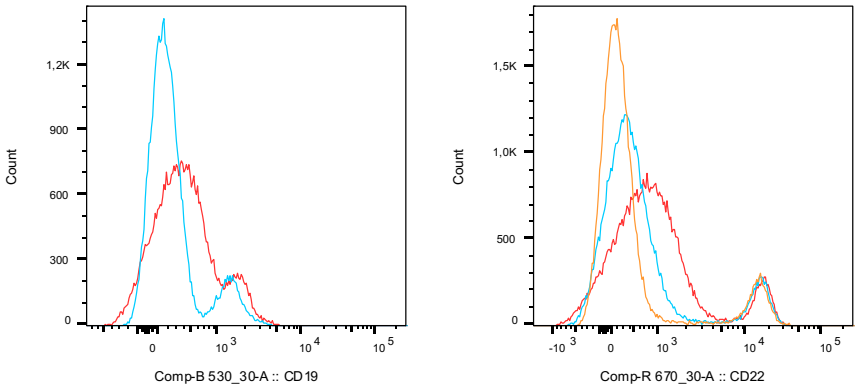
Stain Index of various fluorochrome conjugates on a BD LSR II

Reagent	Clone	Filter	Stain Index
PE-Cy™5[2]	RPA-T4	695/40	353
PE	RPA-T4	585/40	302
APC[1]	RPA-T4	660/20	278
Alexa Fluor® 647[1]	RPA-T4	660/20	214
PE-Cy7	RPA-T4	780/60	139
PerCP-Cy™5.5[2]	RPA-T4	695/40	107
BD Horizon™ V450[5]	RPA-T4	440/40	85
Pacific Blue™[5]	RPA-T4	440/40	80
Alexa Fluor® 488[1]	RPA-T4	530/30	73
Alexa Fluor® 700	RPA-T4	720/45	61
FITC[1]	RPA-T4	530/30	56
APC-Cy7[4]	RPA-T4	780/60	37
PerCP[2]	RPA-T4	695/40	37
AmCyan	RPA-T4	525/40	25
BD APC-H7[4]	RPA-T4	780/60	24

SSOPP 0120

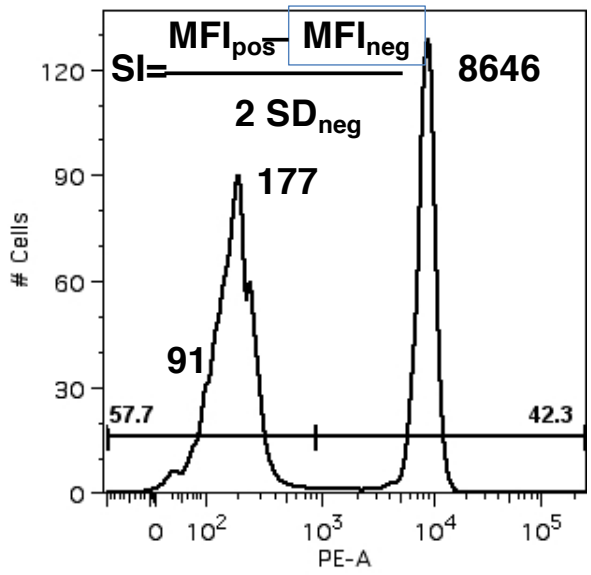
Histogram

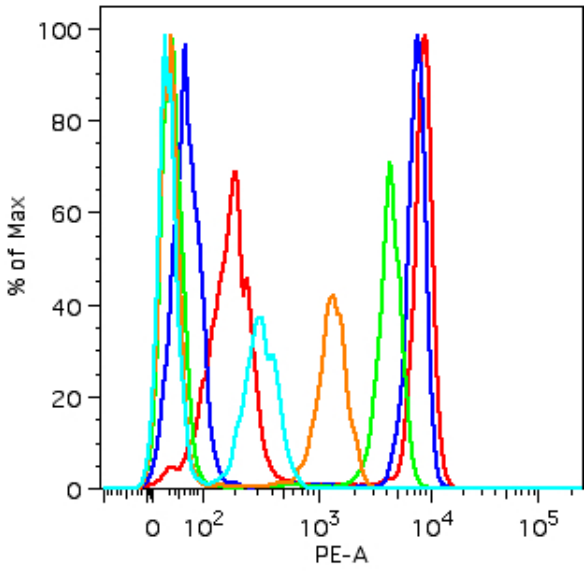




SSOPP 0120

Stain index

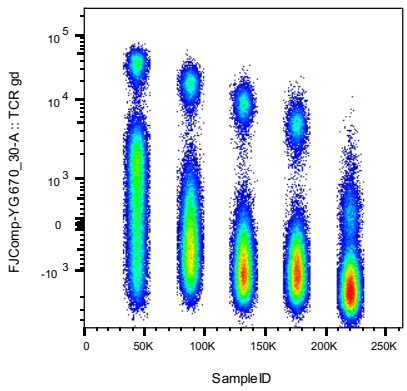




SI=47

SI=47
SI=105
SI=67
SI=31
SI=8

Two dimensional plot



Difficult antigens

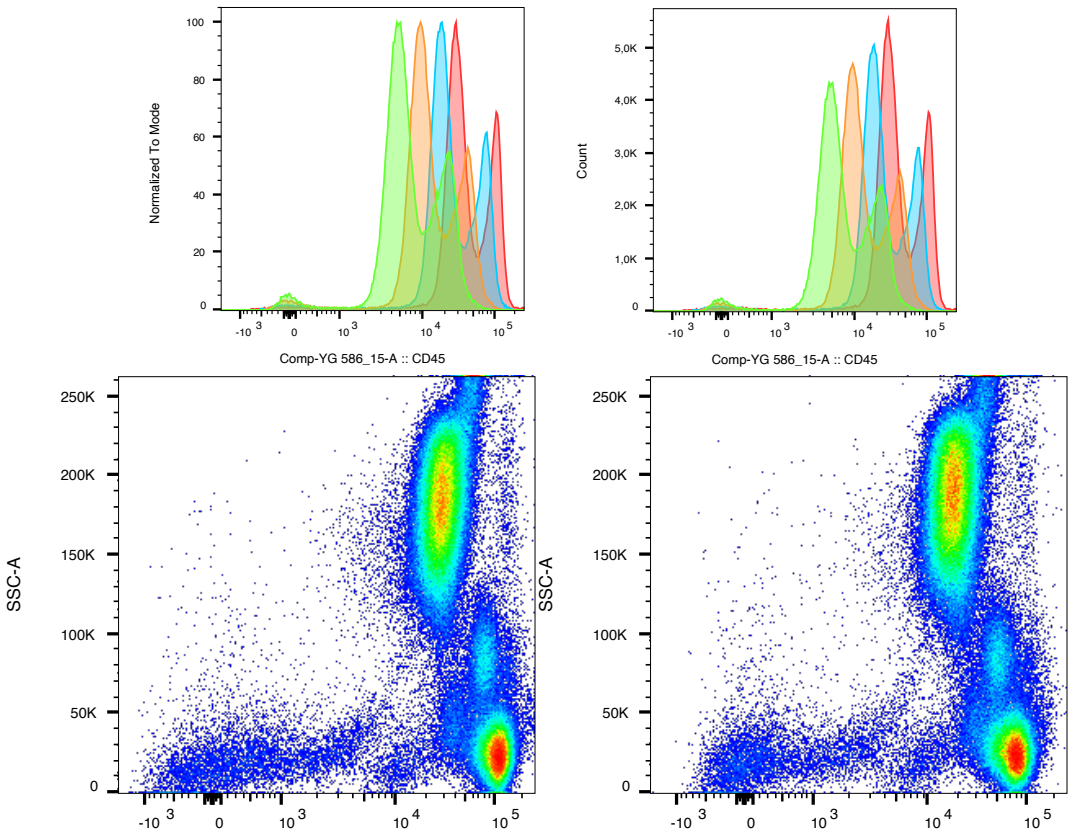
Expressed on all cells

Expressed on few cells

Expressed at low levels

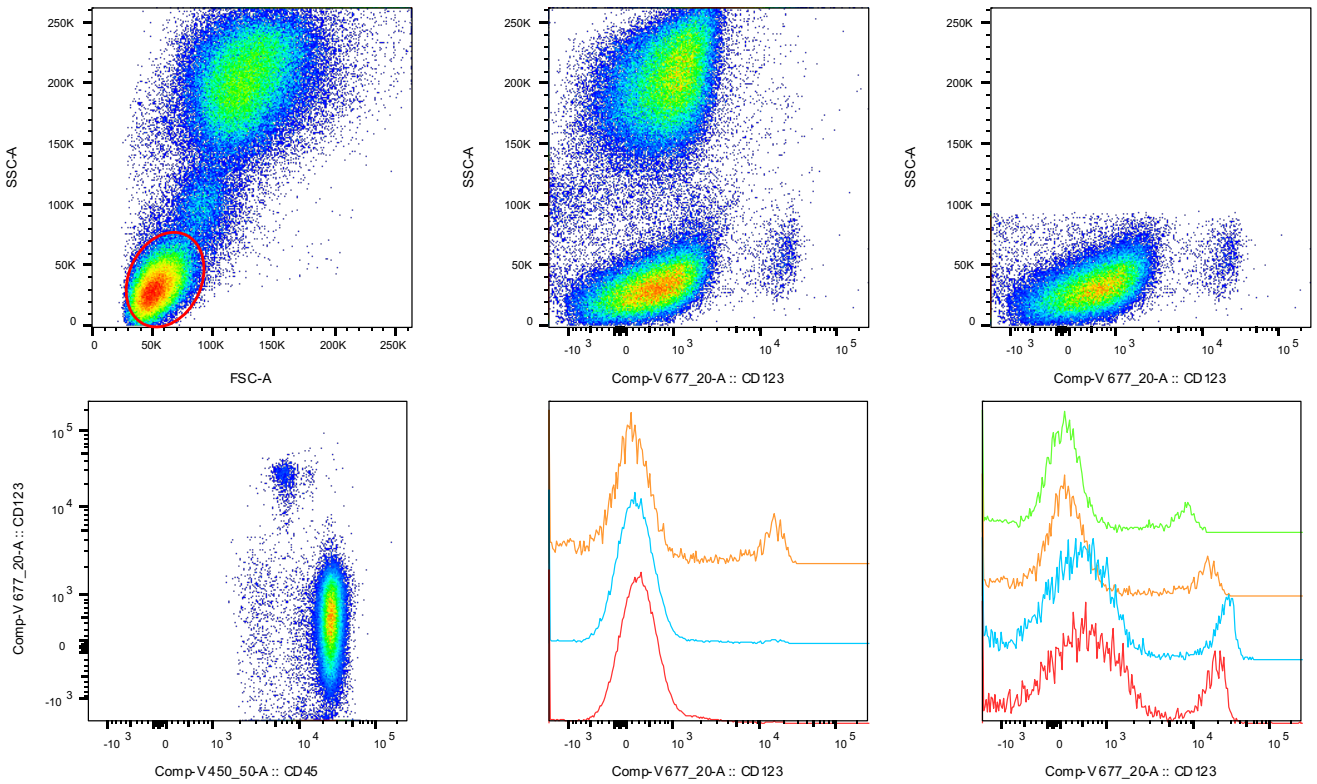
SSOPP 0120

Antigen on all cells



SSOPP 0120

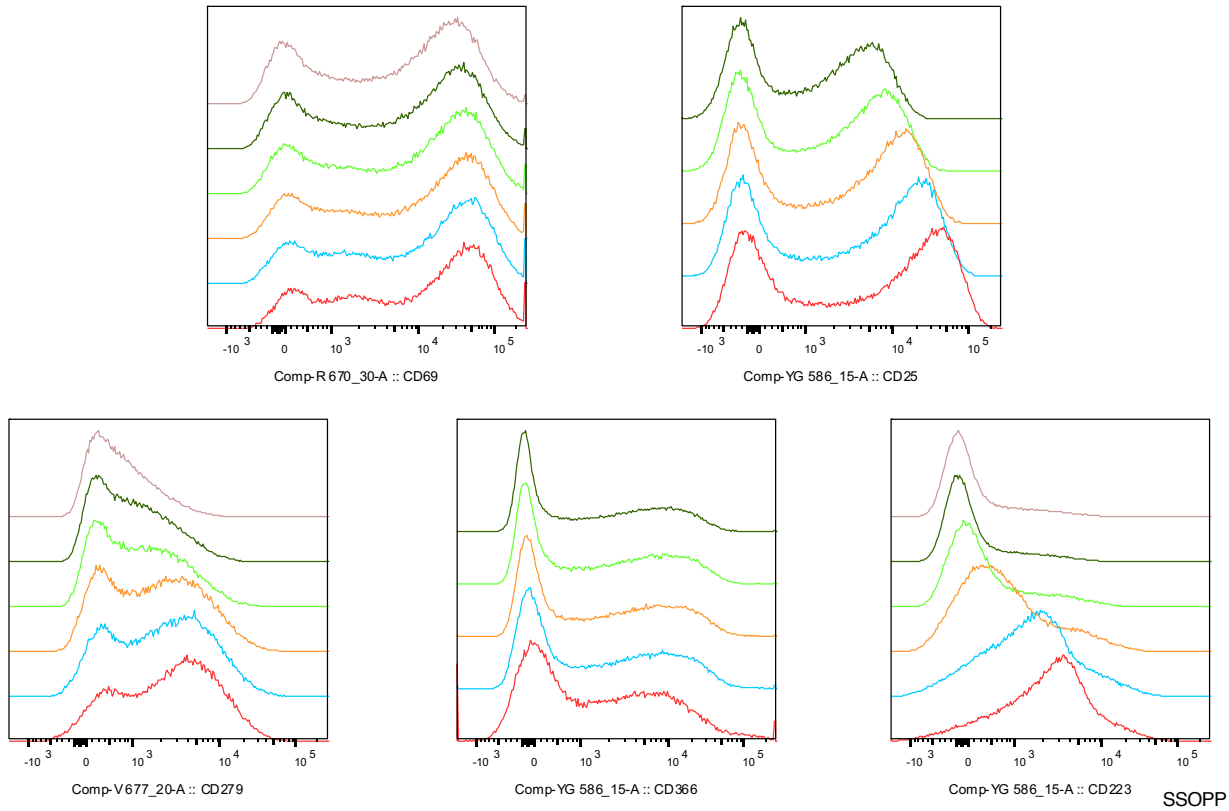
Antigen on small populations



CD123, IL-3 receptor expressed on Basophils and pDC

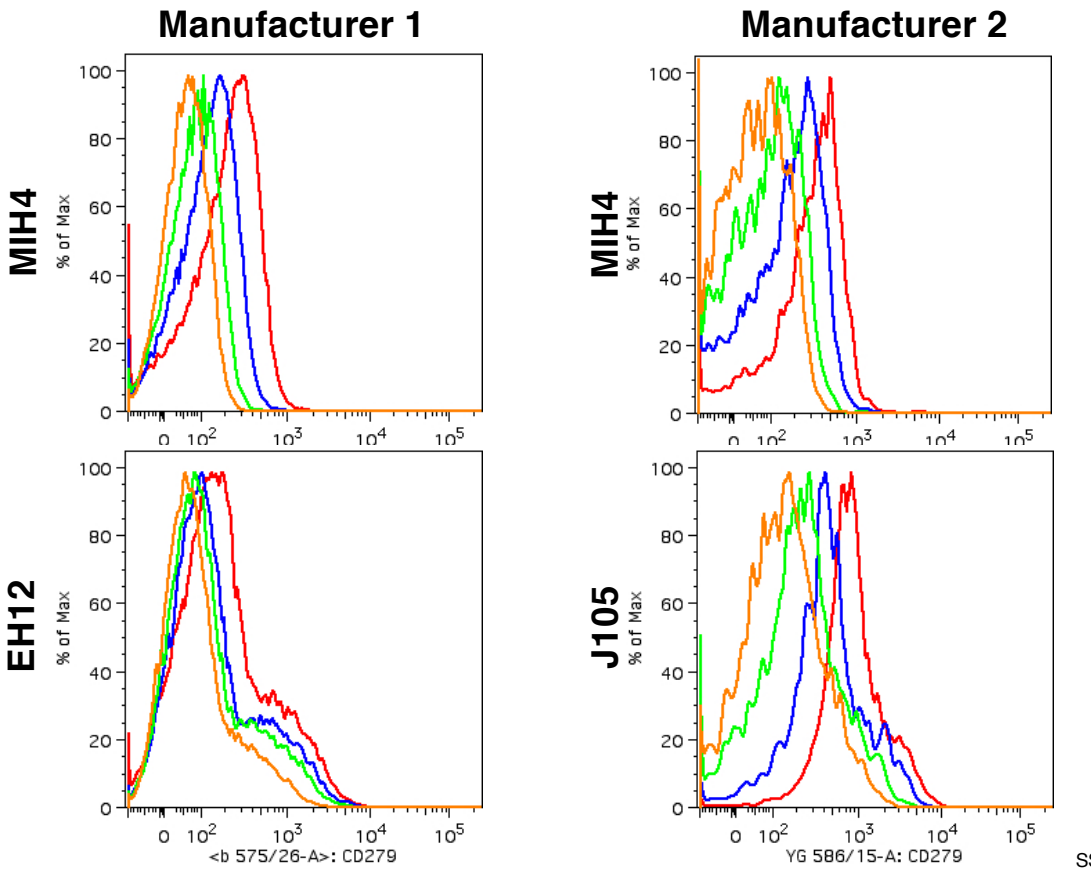
SSOPP 0120

Antigen expressed at low levels



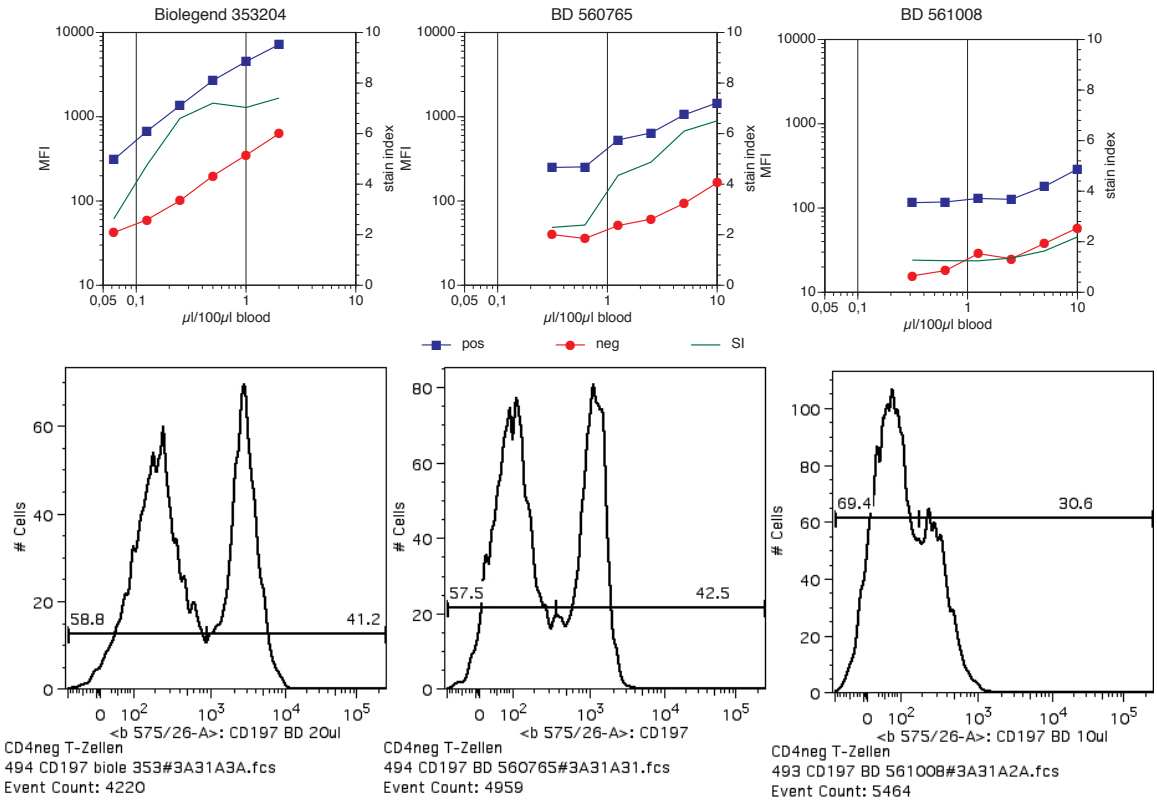
SSOPP 0120

Comparison of clones: CD279



SSOPP 0120

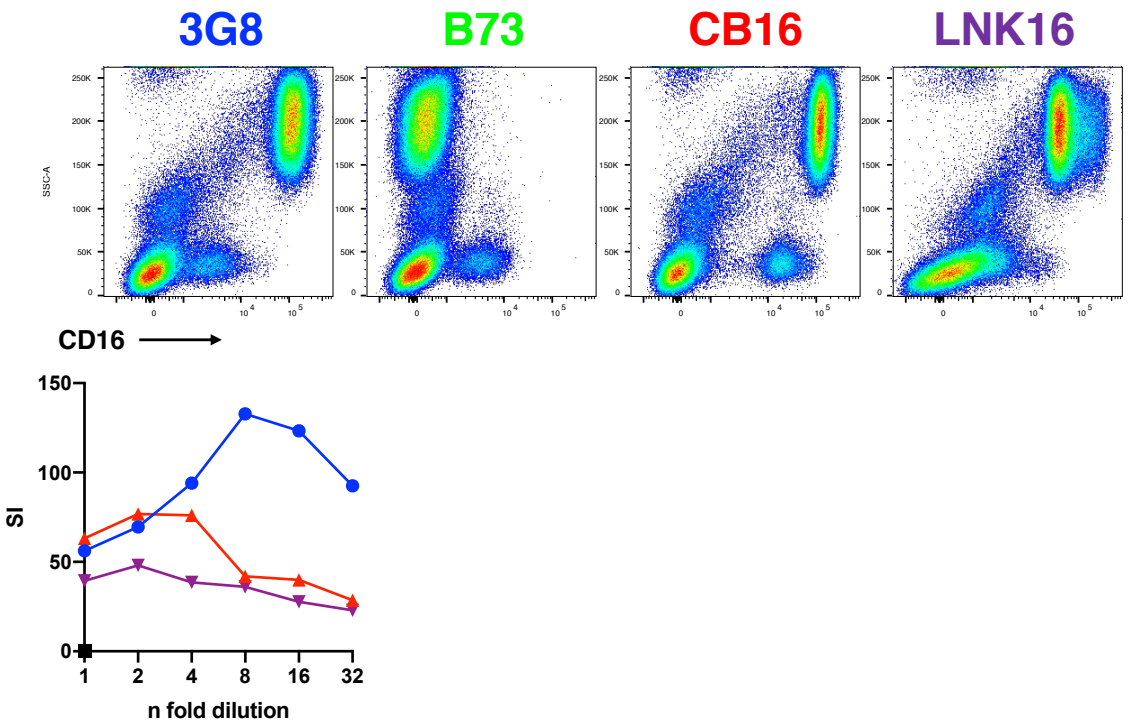
Comparison of clones: CD197



Klon G043H7 Klon 150503 Klon 3D12

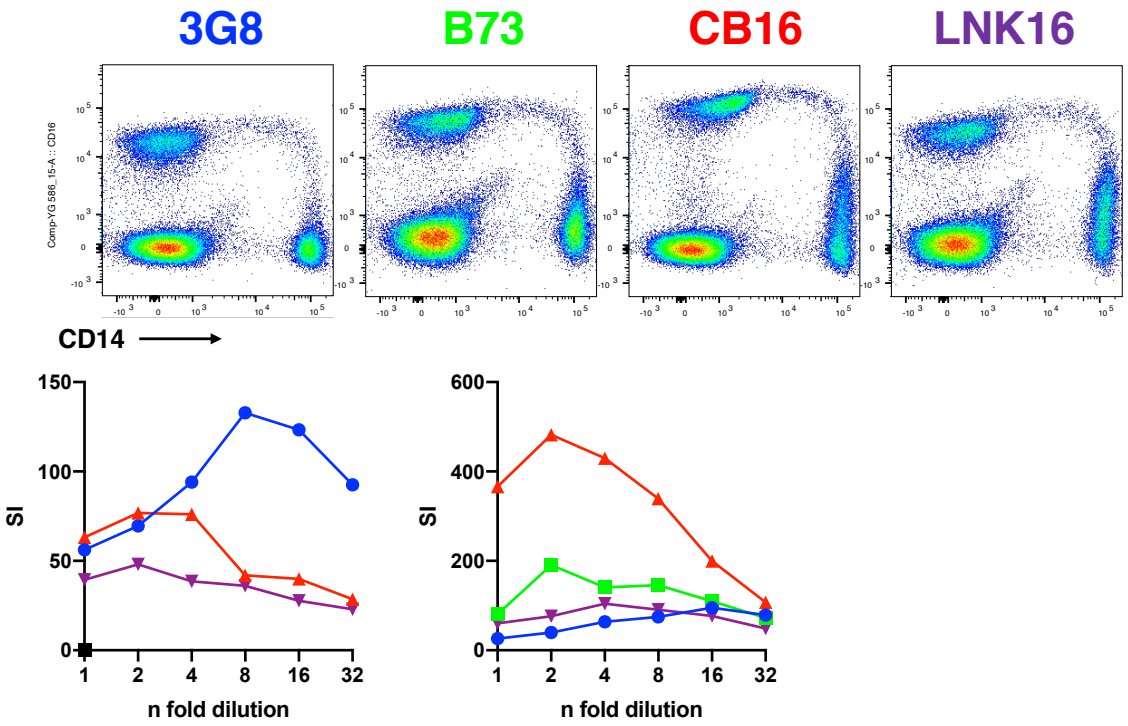
OPP 0120

Whole blood : PBMC



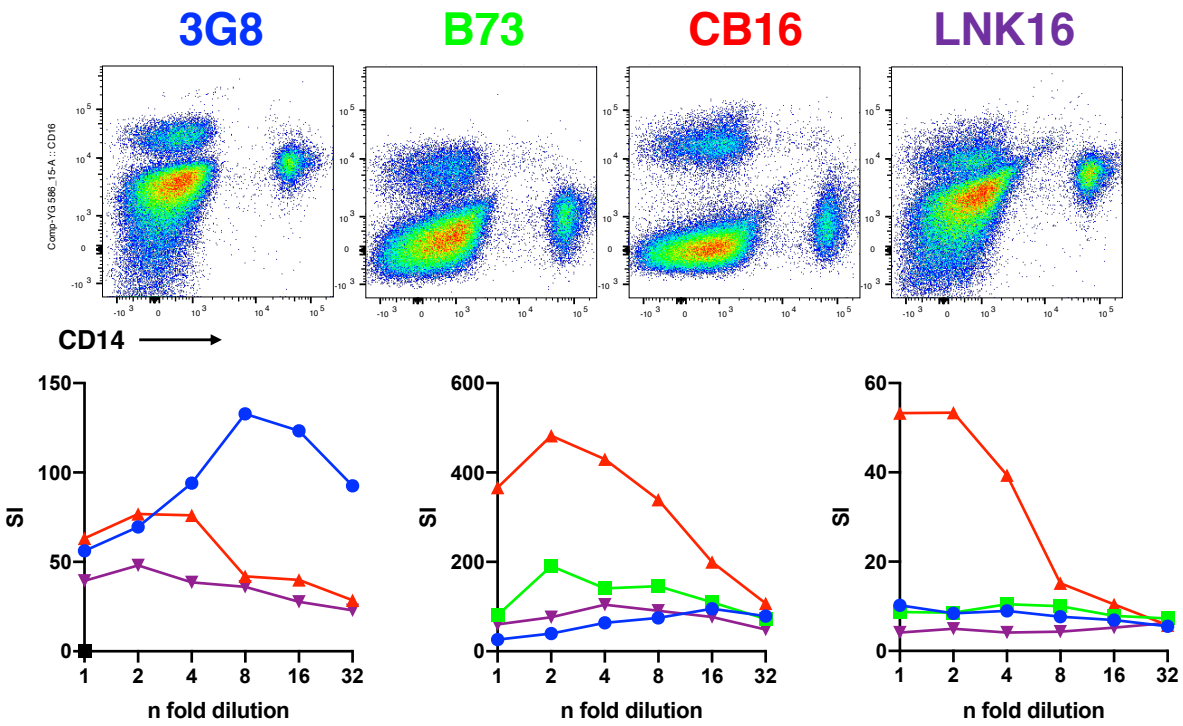
SSOPP 0120

Whole blood : PBMC



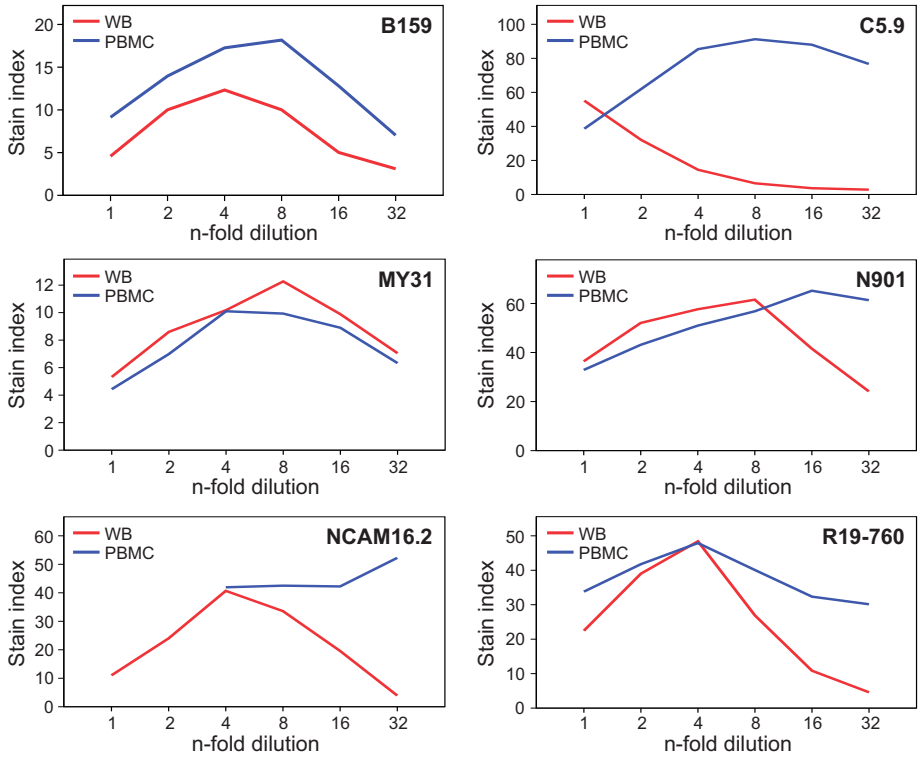
SSOPP 0120

Whole blood : PBMC



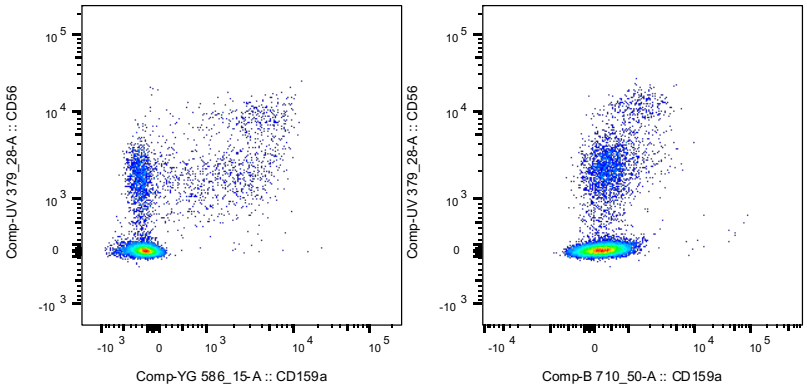
SSOPP 0120

Comparison of clones: CD56

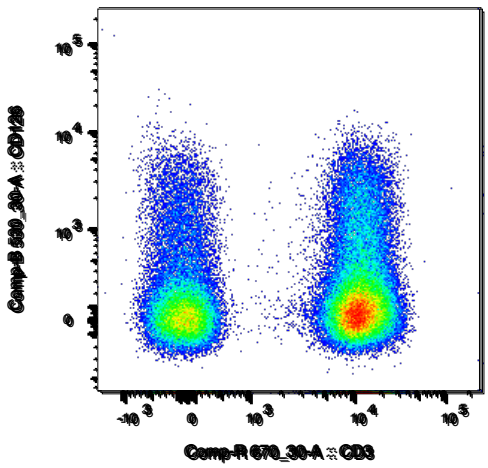


SSOPP 0120

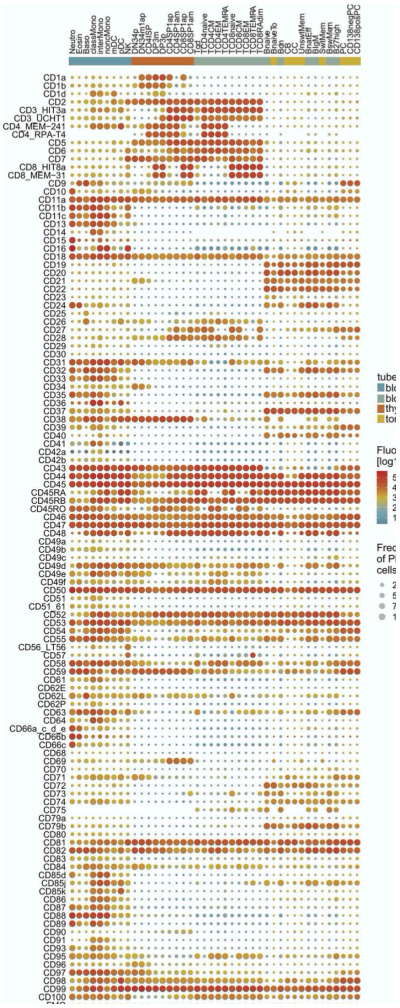
Changing clone and fluorochrome



Surprise, antibody working



Testing antibodies against new



Information on antigen expression

CD lists, information of vendors

Information on staining pattern

information of vendors, publications

Online resource:

https://www.antibodypedia.com/

http://www.hcdm.org

http://bioinformin.cesnet.cz/CDmaps/

Kalina T Front Imm 2019