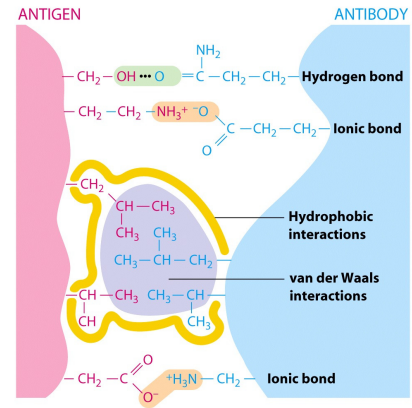


Chapter 6

Antigen-Antibody Interactions: Principles and Applications



Antigen-Antibody Properties

- You must remember Antibody **affinity** (single) VS **avidity** (multiple)
- High affinity: bound tightly and longer!
- Cross-reactivity**: occurs when two different antigens share an identical or very similar epitope. The antibody's affinity for the cross-reacting epitope will be _____ than for the original epitope.
- ABO blood groups and infectious diseases (Streptococcal M antigens, Jenner?)

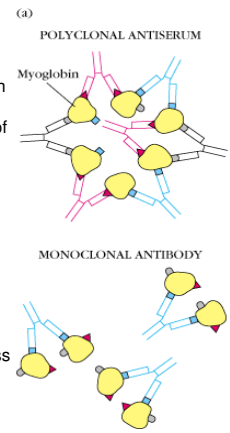
Precipitin reactions

The interaction of antibody with antigen in solution may cause formation of an insoluble lattice that will **precipitate** out of solution.

This precipitate will only form if:

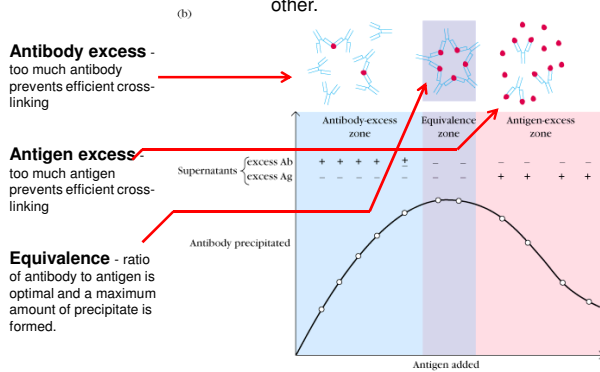
- The antibody is **bivalent** or **polyvalent**
- The antibody or antibody mixture can bind to at least two different sites on the antigen (either two different epitopes or two identical epitopes)

Monoclonal antibodies are likely to be less efficient at immunoprecipitation than polyclonal antibodies.



Kuby Figure 6-4a

Formation of the precipitate also requires that the antigen and antibody be present at appropriate concentrations relative to each other.



Kuby Figure 6-4b

TABLE 6-3 Sensitivity of various immunoassays

Assay	Sensitivity* (µg antibody/ml)
Precipitation reaction in fluids	20-200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10-50
Ouchterlony double immunodiffusion	20-200
Immunoelectrophoresis	20-200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	0.006-0.06
Agglutination inhibition	0.006-0.06
Radioimmunoassay (RIA)	0.0006-0.006
Enzyme-linked immunosorbent assay (ELISA)	-0.0001-0.01
ELISA using chemiluminescence	-0.00001-0.01†
Immunofluorescence	1.0
Flow cytometry	0.006-0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.
 †Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.
 SOURCE: Updated and adapted from N.R. Rose et al., eds., 1997. *Manual of Clinical Laboratory Immunology*, 5th ed., American Society for Microbiology, Washington, DC.

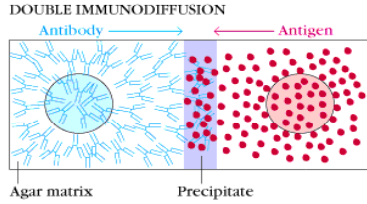
Table 6-3
 Kuby IMMUNOLOGY, Sixth Edition
 © 2007 W.H. Freeman and Company

Double Immunodiffusion (Ouchterlony)

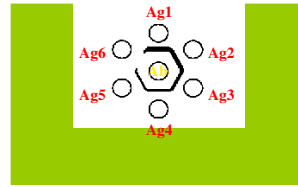
Diffusion of antibody and antigen towards each other in an agarose gel.

A line of precipitate will form if the antibody binds to antigen.

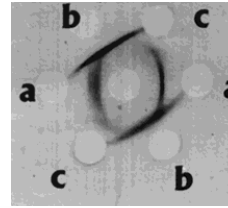
Used to determine if an antigen or antibody is present.



Kuby Figure 6-5



<http://www.fbr.org/swksweb/immunol1.html>



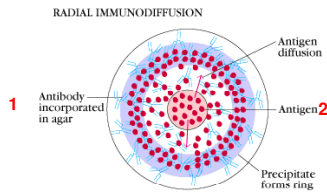
Volume 271, Number 30, Issue of July 26, 1996 pp. 18054-18060 JBC

Radial Immunodiffusion

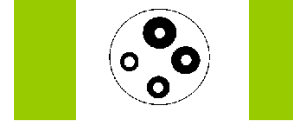
Diffusion of antigen through an agarose gel containing antibody.

A precipitin ring will form. The size of the ring is proportional to the antigen concentration.

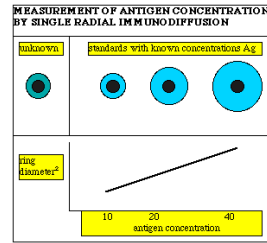
Used to determine concentrations of specific proteins (low sensitivity)



Kuby Figure 6-5



<http://www.fbr.org/swksweb/immunol1.html>



<http://leah1.kcc.hawaii.edu/~jahn/micro/medmicro/medmicro.11.html>

Hemagglutination

Antibody can also cross-link cells or beads.

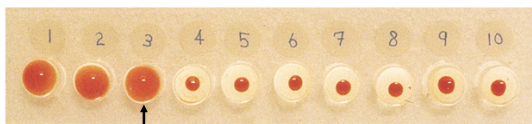
Cross-linking of red cells is called hemagglutination.

Non-cross-linked cells settle in a bead to the bottom of the well.

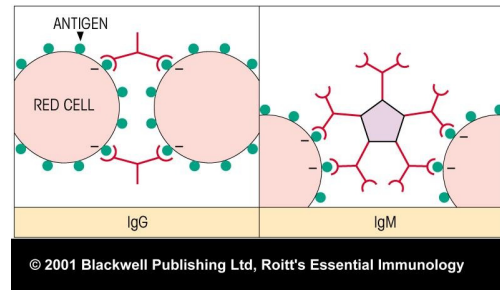
Cross-linked cells settle in a diffuse pattern.

Used to measure antibody presence and level (titer).

Used to measure antibodies to red cell antigens or to other antigens bound to the surface of red cells.



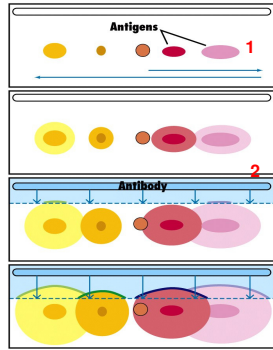
Kuby Figure 6-8



© 2001 Blackwell Publishing Ltd, Roitt's Essential Immunology

Figure 6.7

Immunelectrophoresis



1. Antigens are electrophoresed to separate them based on their charge
2. A trough is cut and antiserum is added.

Figure 6-7
Auby IMMUNOLOGY, Sixth Edition
© 2007 W.H. Freeman and Company

Enzyme-linked immunosorbent assay (ELISA)

Used to measure antigen or antibody presence and concentration.

Far more sensitive than precipitin or agglutination techniques.

Relies on the ability to covalently conjugate chemicals to the Fc region of Ig without interfering with antigen binding ("enzyme-linked") and the ability of plastic to nonspecifically bind proteins (immunosorbent).

In ELISA, an enzyme is bound to the Fc region - usually horseradish peroxidase or alkaline phosphatase. Enzyme presence can be determined by use of colorimetric substrates.

Final measurement is an absorbance.

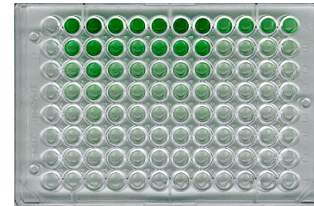
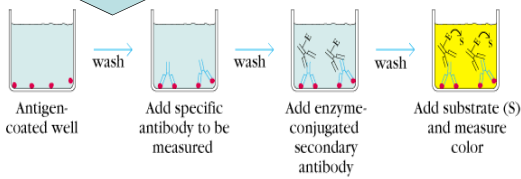
Comparison with standard curves indicates concentration of antigen or antibody.

Various assay formats are possible.

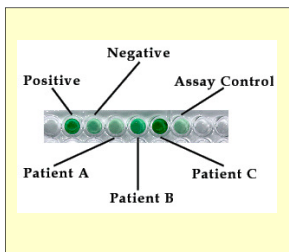


BLOCKING is very important!!!

(a) Indirect ELISA



<http://www.supercolosturum.com/colosturum/information/information9.htm>

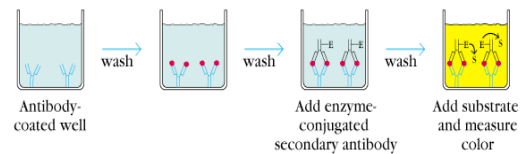


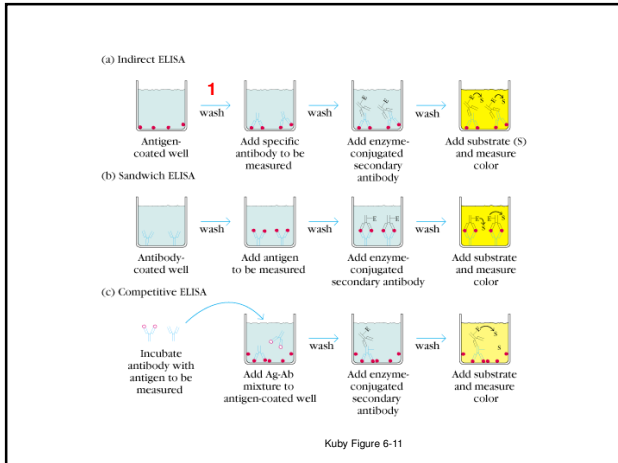
Sample	Absorbance
Positive control	1.689
Negative control	0.153
Assay control	0.123
Patient A	0.055
Patient B	0.412
Patient C	1.999

<http://www.supercolosturum.com/colosturum/information/information9.htm>



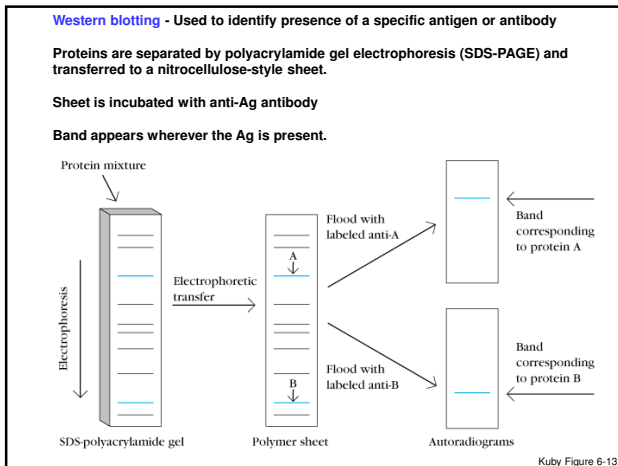
(b) Sandwich ELISA





Relative sensitivities:

Precipitin reactions < Agglutination reactions < ELISA

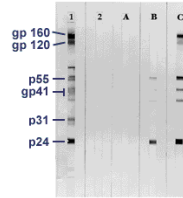


HIV Western Blot

No bands presentNegative

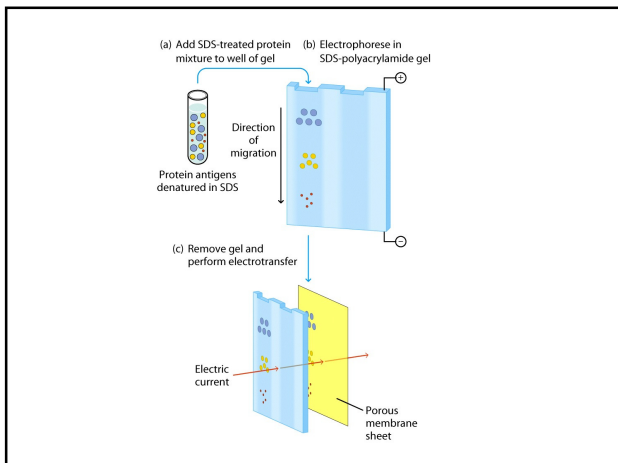
Bands at either p31 OR p24 AND bands present at either gp160 OR gp120Positive

Bands present, but pattern does not meet criteria for positivityIndeterminate



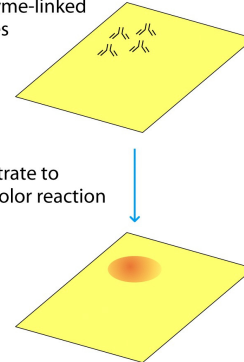
- Lane 1, HIV+ serum (positive control)
- Lane 2, HIV- serum (negative control)
- Lane A, Patient A
- Lane B, Patient B
- Lane C, Patient C

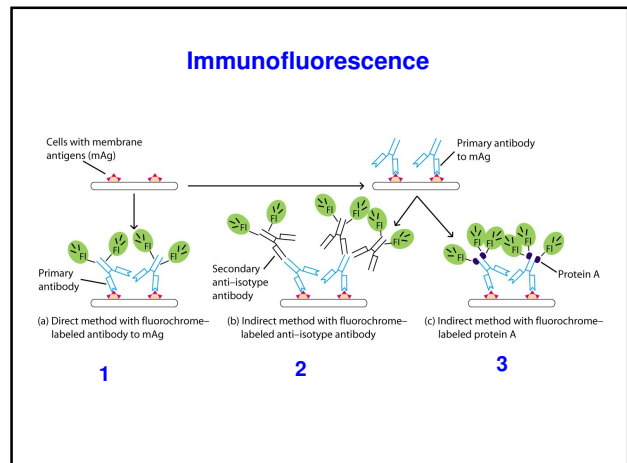
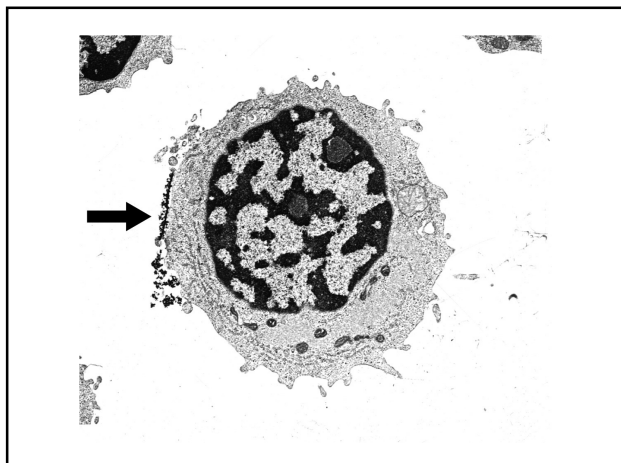
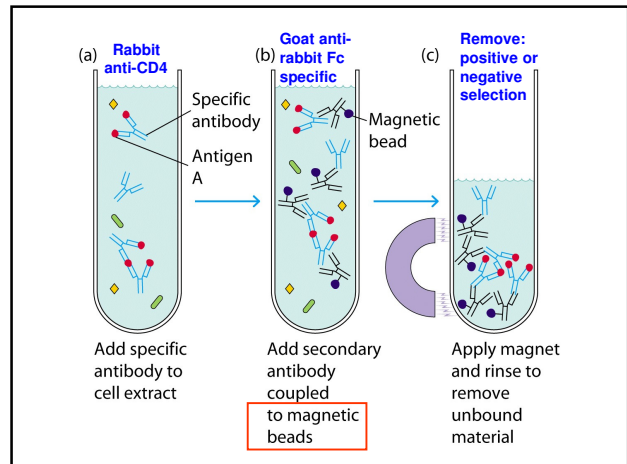
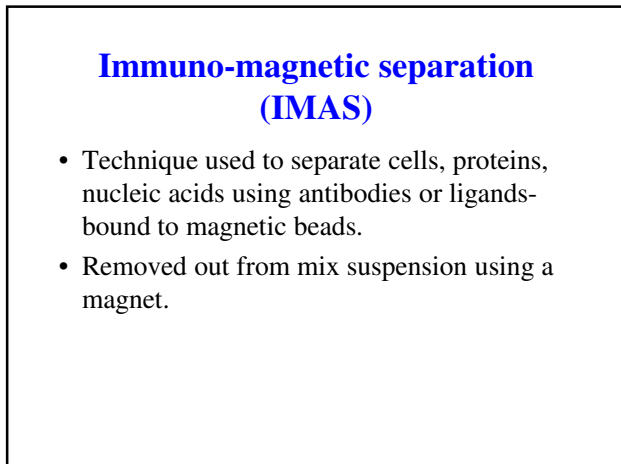
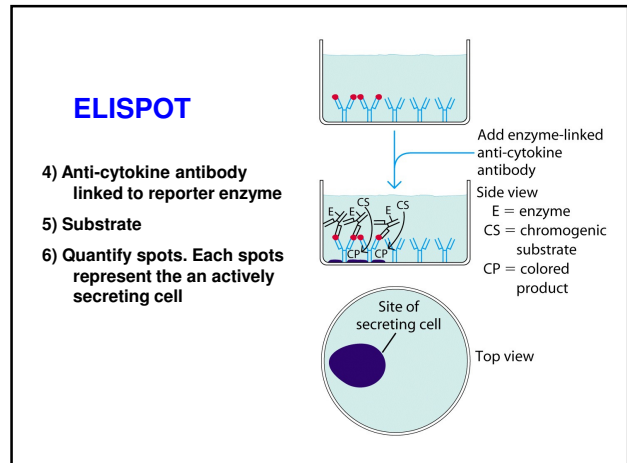
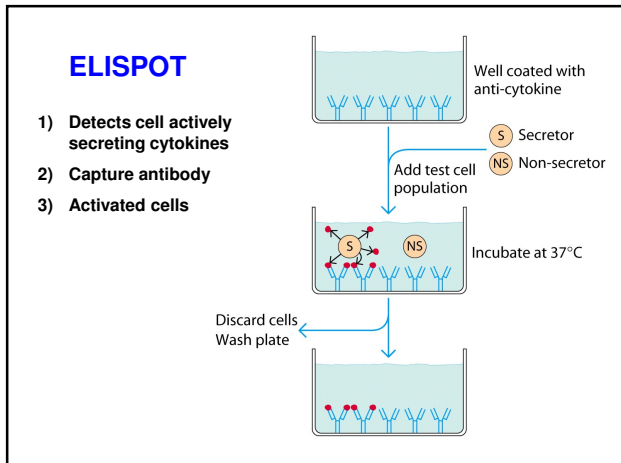
http://www.biology.arizona.edu/immunology/activities/western_blot/west2.html

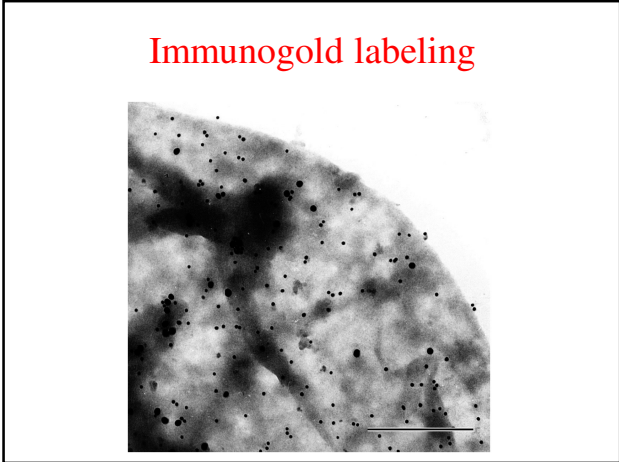
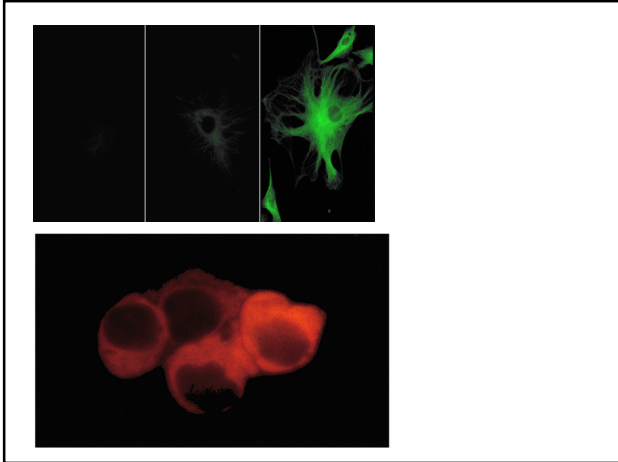


(d) Bind antigen of interest with enzyme-linked antibodies

(e) Add substrate to activate color reaction

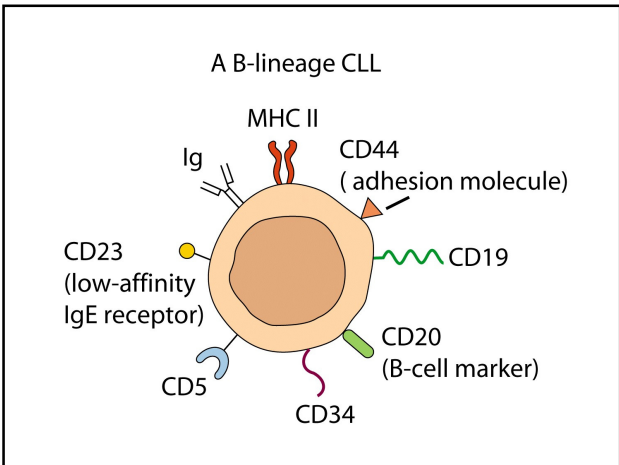
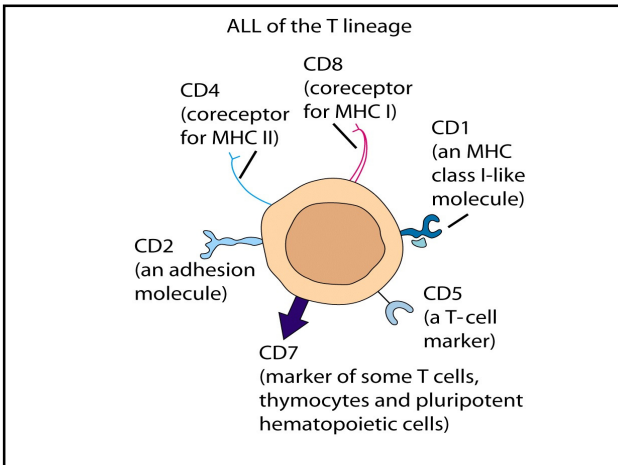
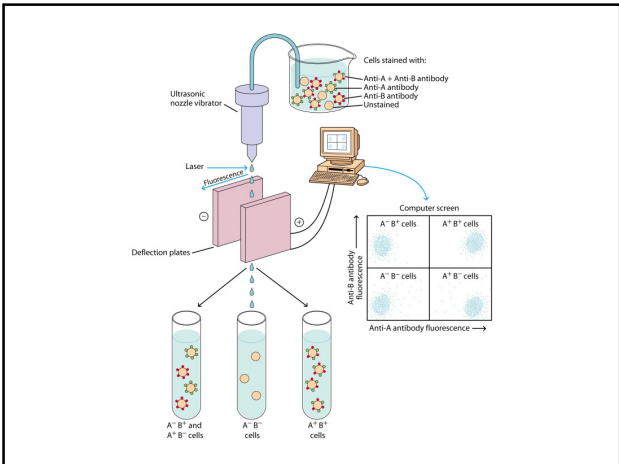






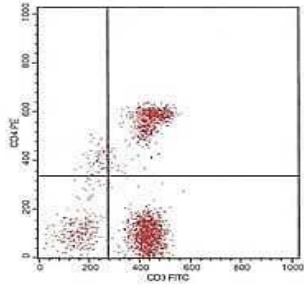
Fluorescence Activated Cell Sorter (FACS)

- Mixed cell population
- Requires two different fluorochromes
- Commonly used: FITC, PE, Texas Red, etc



FACS Analysis

Reagents:
CD4-PE Phycoerythrin (PE)
VS
CD3 Fluorecein (FITC)

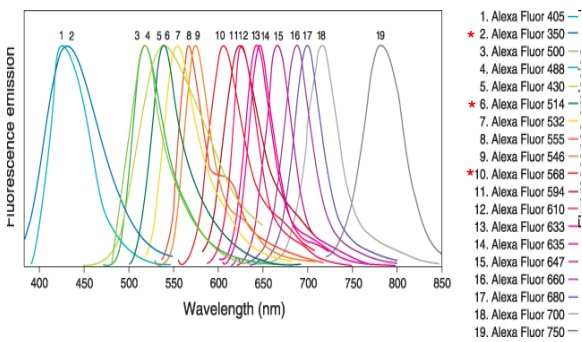


Markers for Th cells

- **Naïve** – CD62L (L-selectin), CD45RA
- **Activated** – CD25, CD69, CD38
- **Memory** – CD45RO, CD44

High (hi) vs Low (lo)

The Alexa Fluor Dye Series



Commonly Used Fluorochromes

Probe	Ex (nm)	Em (nm)	MW
Reactive and conjugated probes			
Cascade Blue	375;400	423	596
Lucifer yellow	425	528	
Phycoerythrin (PE)	480;565	578	240 k
Fluorescein (FITC)	495	519	389
BODIPY-FL	503	512	
TRITC	547	572	444
Rhodamine	570	576	548
Texas Red	589	615	625
TruRed	490,675	695	

NAME	PARTNER	ASSIGNMENT
Barbano,Duane	Bollig, Molly Calypso	Innate Immunity
Campbell,Natalie Elaine	Schult, Deborah Ann	Acquired Immunity
Chapman,Carl L		Innate Immunity
Christensen,Lanette M	Martin, Lily	Innate Immunity
Cole,Elizabeth A	True, Sarah R.	Innate Immunity
Cooper,Paige Ashley	Diamond, Ayla R	Acquired Immunity
Cousineau Cote,Vincent		Innate Immunity
Dele,Jerilyn Jean		Innate Immunity
Doles,Jennifer L		Innate Immunity
Dooda,Nicole Briana	Stupey, Trevor Matthew	Innate Immunity
Enriquez,Amber Nicole	Frank, Jessica N	Acquired Immunity
Gifford,Brian	Wheeler, Sarah M	Acquired Immunity
Gilliland,Morgan Richard	Valentine, Michael C	Acquired Immunity
Grant,James Harvey	Grudman, Heather Lynn	Acquired Immunity
Hayer,Michaela	Juarez, Guillermo	Innate Immunity
Hiebert,Leah Christine	Leshen, Michael A.	Acquired Immunity
Jasillo,Maria Helena	Saucedo, Eric S	Innate Immunity
Jarvis,Kelley Anne		Innate Immunity
Kimberly,Morgan Foster	Zuchowski, Adam	Acquired Immunity
McElroy,Kimberly Marie	Schutt, Mike John	Acquired Immunity
Nurminen,Meljo Petteri	Vanoncini, Stefania	Innate Immunity
Pugh,Garrett Miles	Mentjox, Alyssa M	Innate Immunity
Ramirez,Claire Annabelle		Acquired Immunity
Rosbrook,Spencer Wesley		
Ward		Acquired Immunity
Wasserman,Marc	Willey,Anne Lynn	Innate Immunity
Yazzie,Filbert	Stant,Nyssa Marie	Innate Immunity