Immunological methods

10.06.2026

Carlos Plaza Sirvent

RUHR
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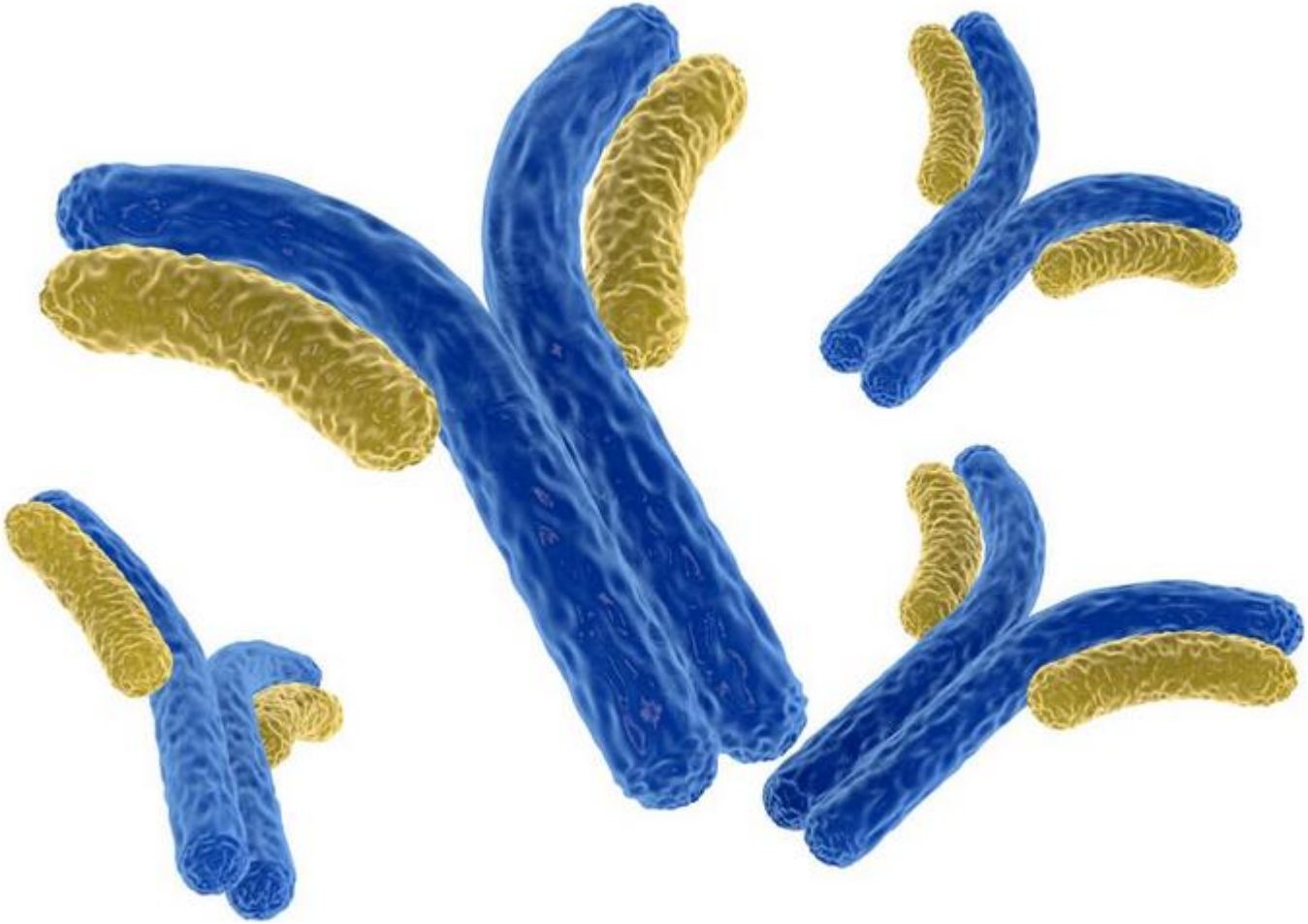
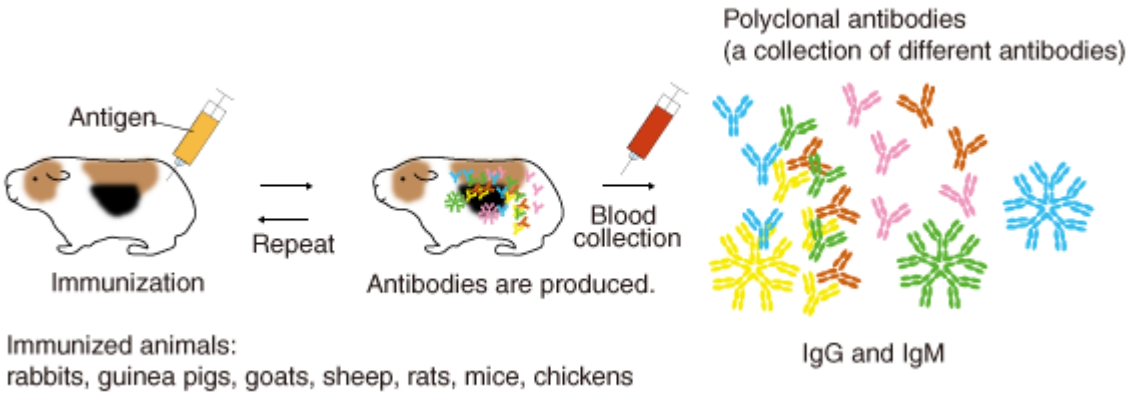
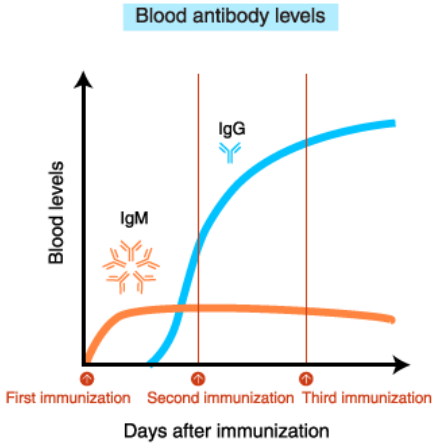
RUB

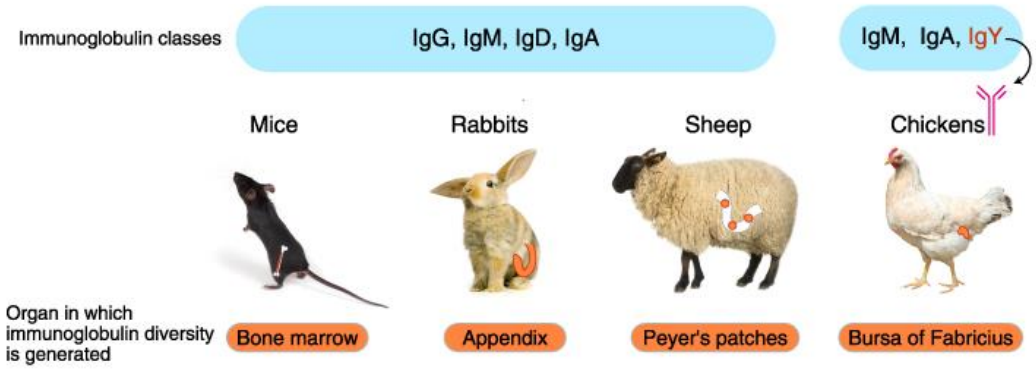
Molecular Immunology

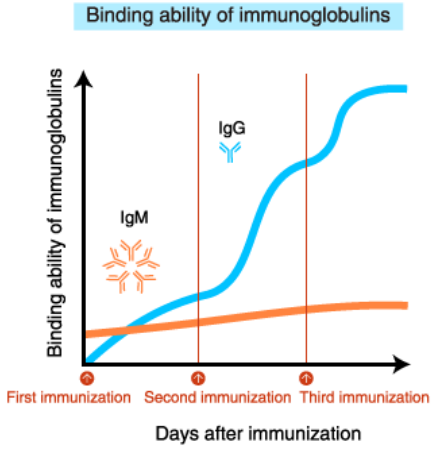


Polyclonal antibody generation









Monoclonal antibodies

The Nobel Prize in Physiology or Medicine 1984

Photo from the Nobel Foundation archive.

Niels K. Jerne

Prize share: 1/3

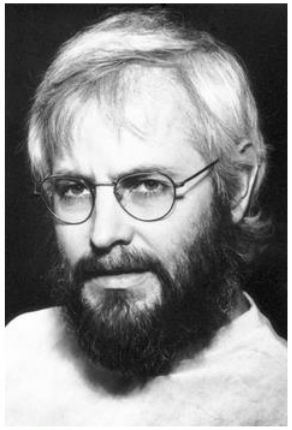


Photo from the Nobel Foundation archive.

Georges J.F. Köhler

Prize share: 1/3

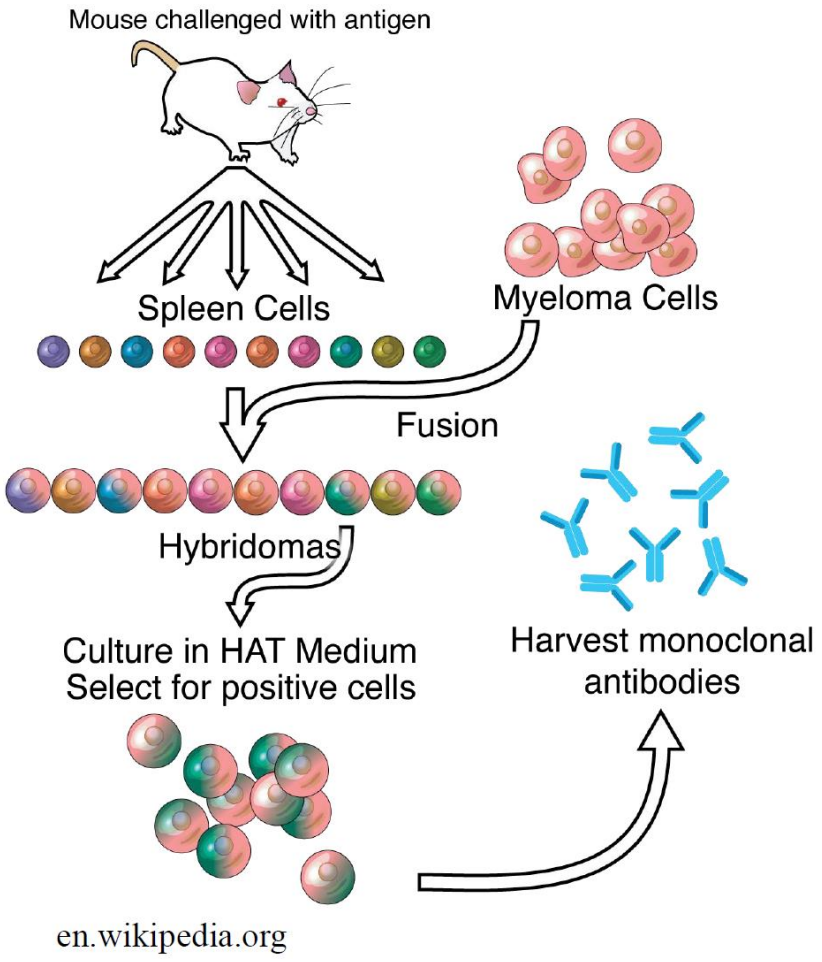
Photo from the Nobel Foundation archive.

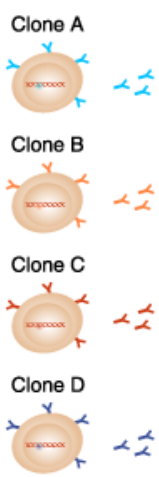
César Milstein

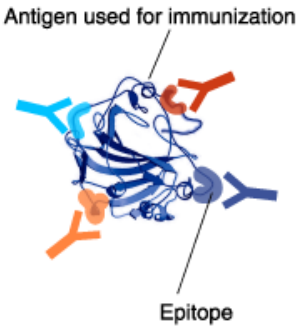
Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1984 was awarded jointly to Niels K. Jerne, Georges J.F. Köhler and César Milstein "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"

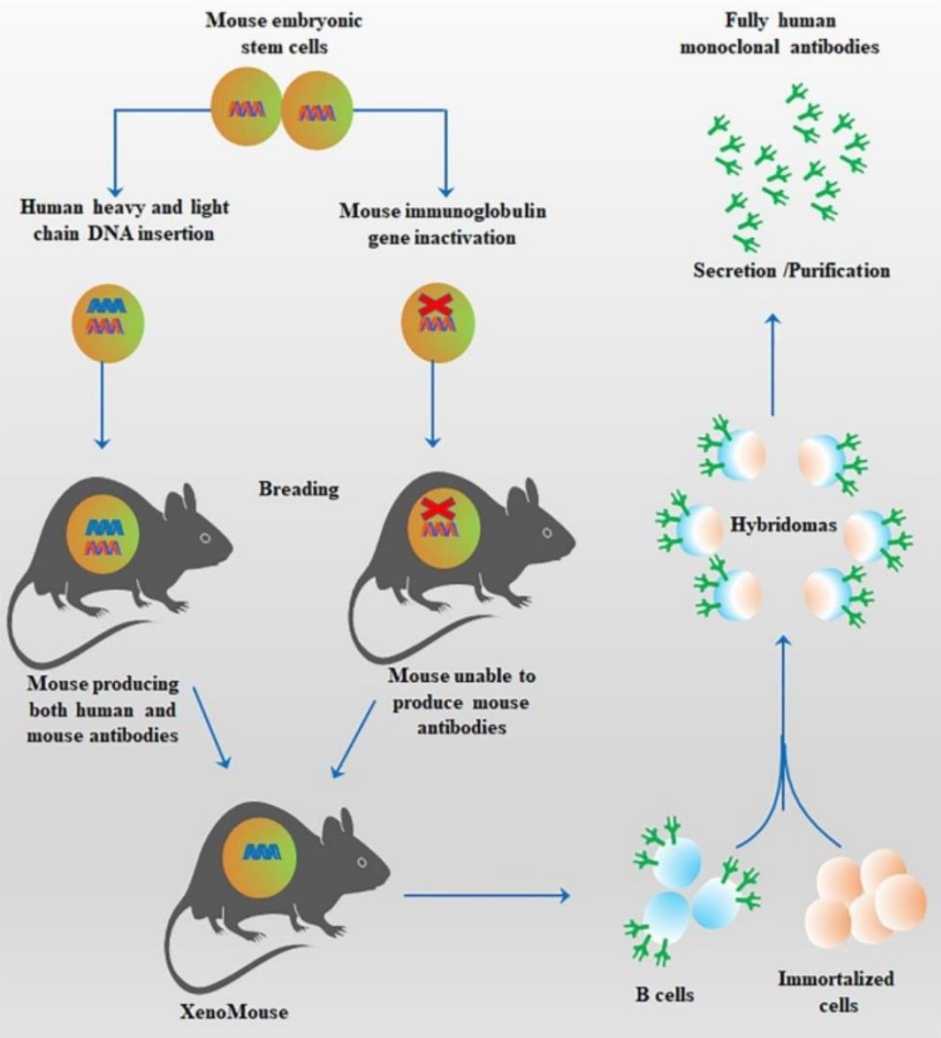
Monoclonal antibody generation





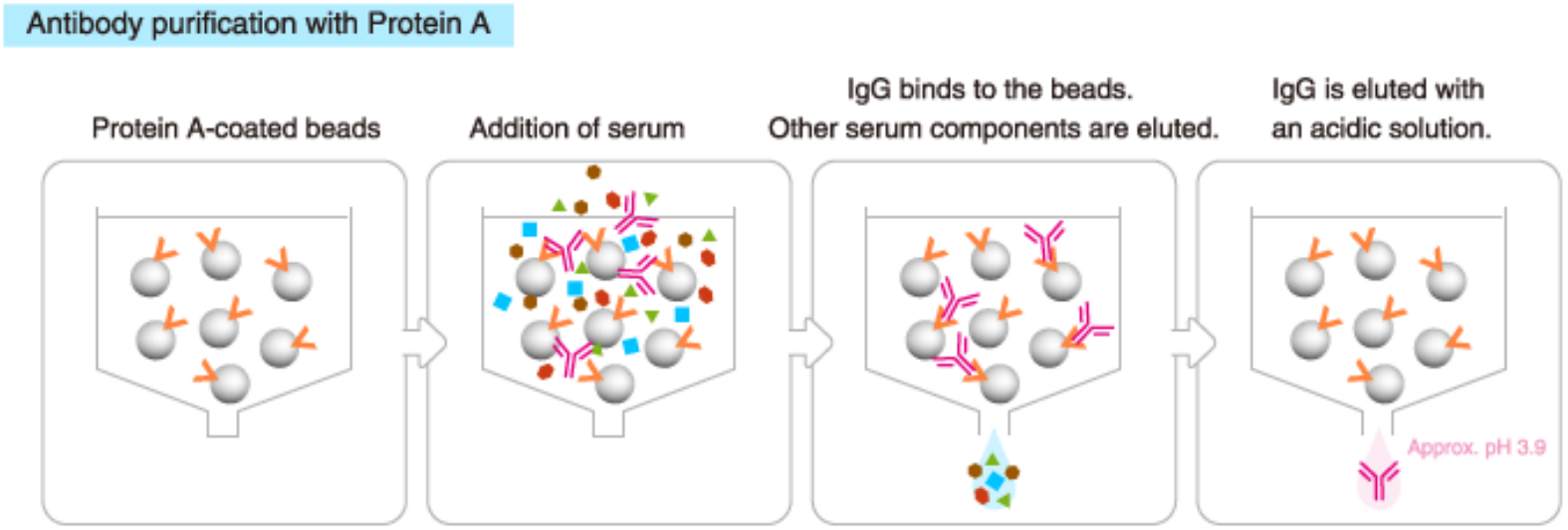


Humanized monoclonal antibody generation

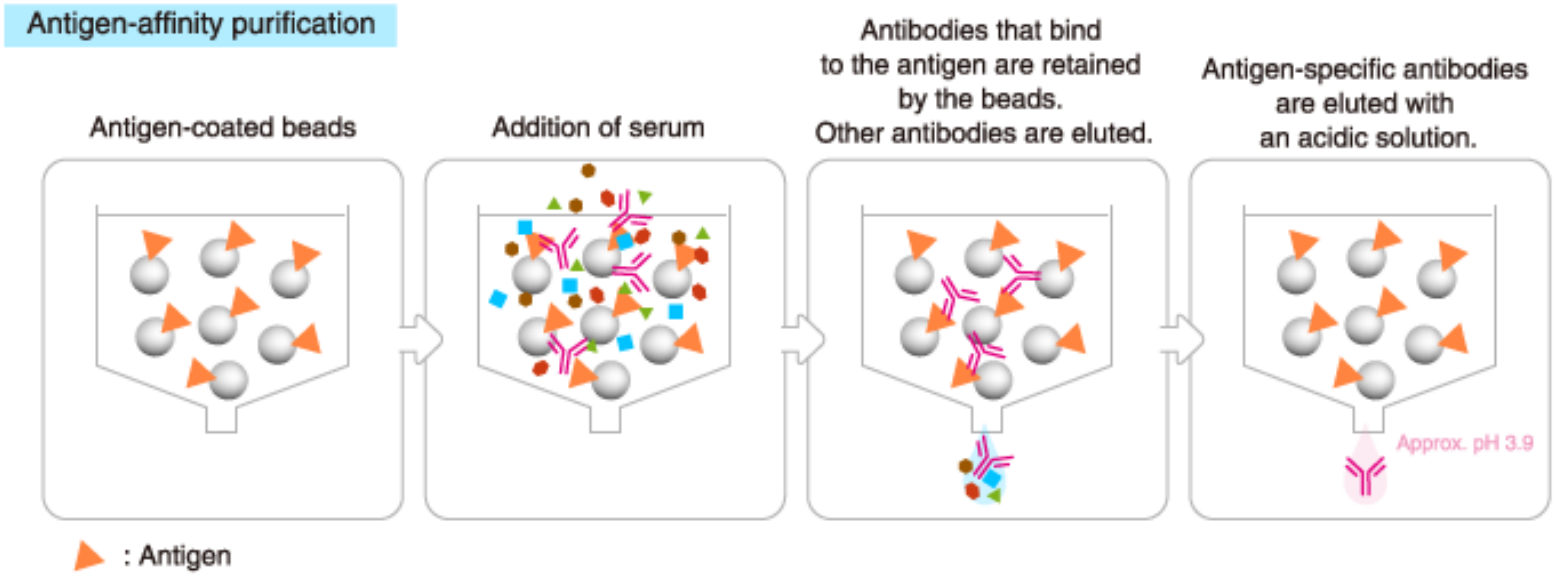


Parray et al., 2020

Antibody purification

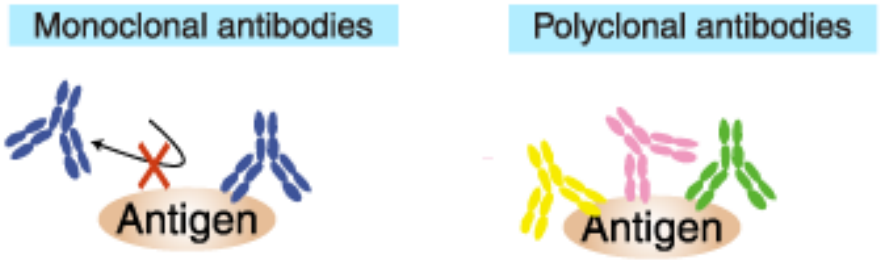


Antibody purification

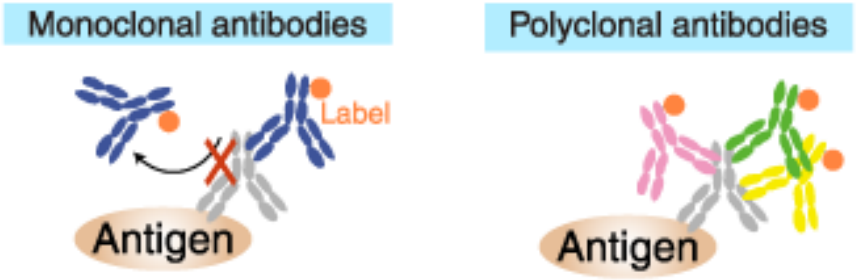


Polyclonal vs. Monoclonal

As primary:



As secondary:

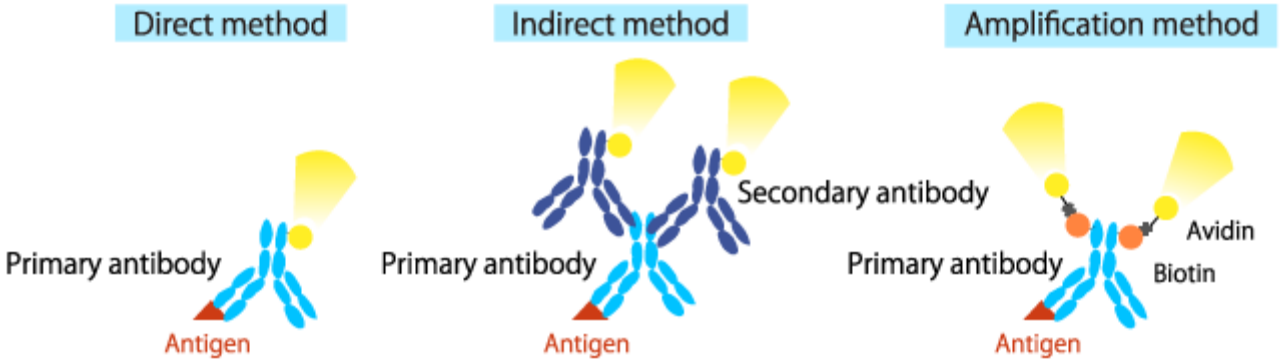


Polyclonal vs. Monoclonal

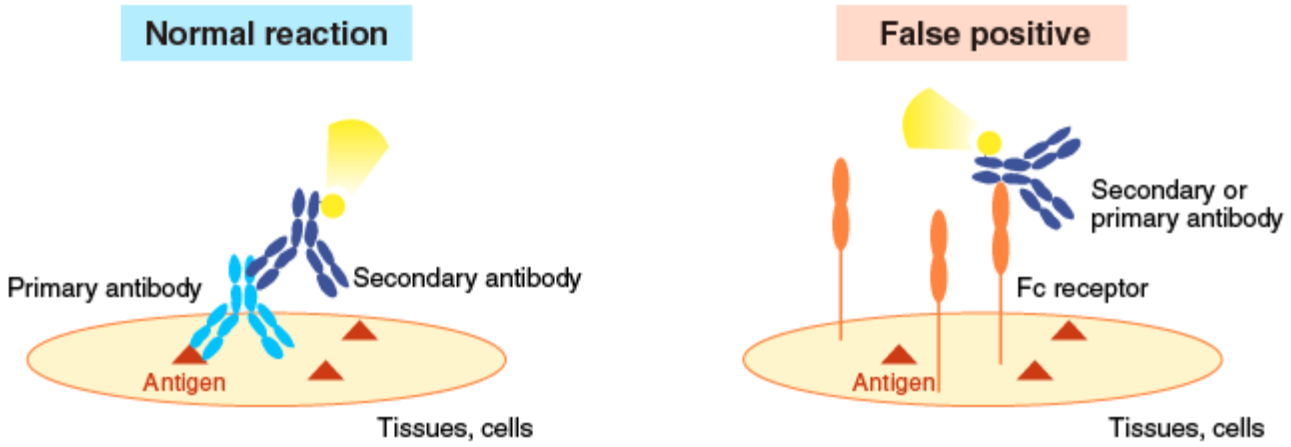
Difference between polyclonal and monoclonal antibodies

	Polyclonal antibodies	Monoclonal antibodies
Animal species	Rabbit, guinea pig, goat, sheep, rat, mouse, chicken, etc.	Rat, mouse, chicken, rabbit, human, etc.
Form	Antiserum	Hybridoma
Class, subclass	Mixed classes	Single class
Epitope	React to multiple epitopes	React to a single epitope
Specificity	Lower than monoclonal antibodies because multiple types of antibodies are present.	High if good quality antibodies are selected.
Reproducibility	Variable among lots.	The same antibodies are produced indefinitely.
Stability	Binding ability tends to be unaffected by fixation/denaturation of the antigen, because multiple different antibody molecules are present. Tolerate modifications, such as labeling and removal of the Fc region.	Binding ability may be lost if the epitope is lost by fixation/denaturation of the antigen, because monoclonal antibodies are homogeneous. Tend to be sensitive to modifications, such as labeling and removal of the Fc region.

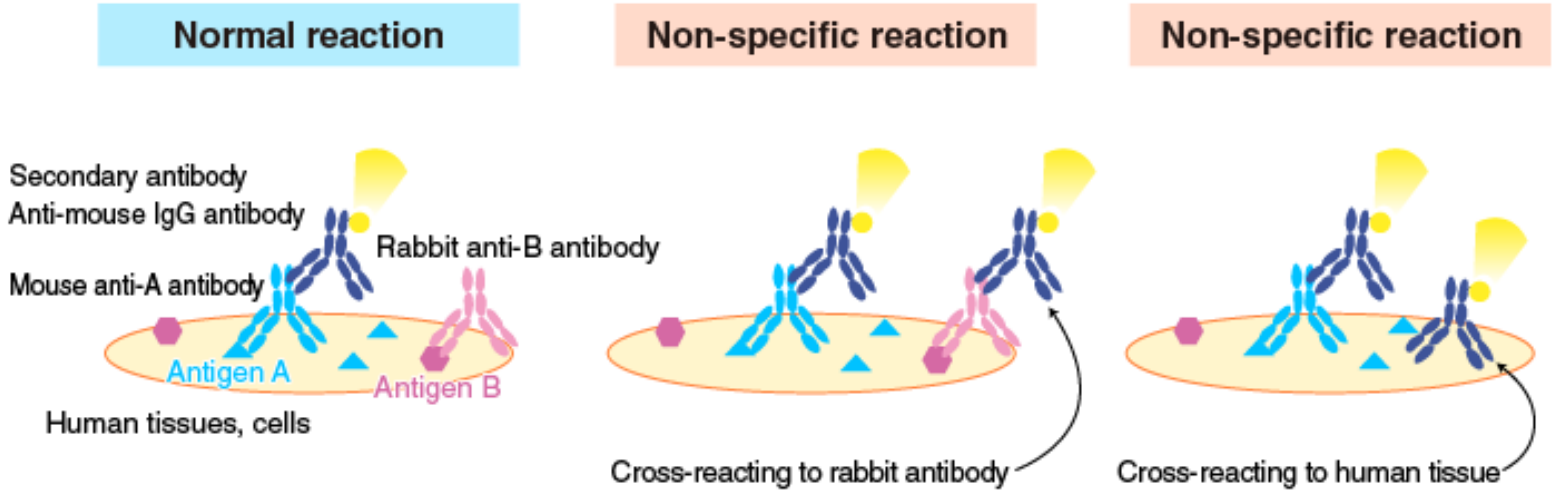
Labeling options



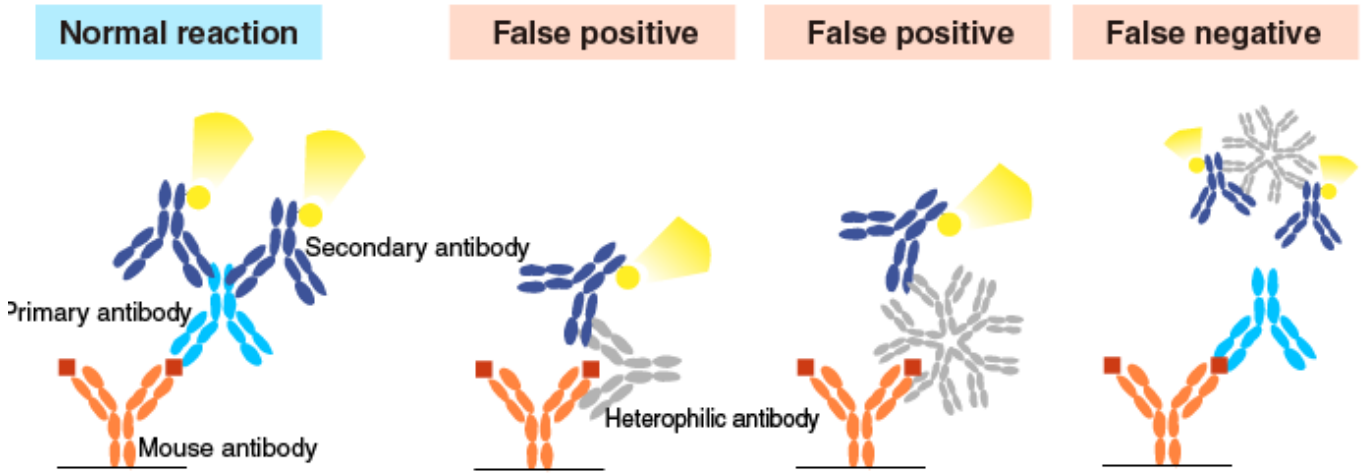
Non-specific reactions



Non-specific reactions

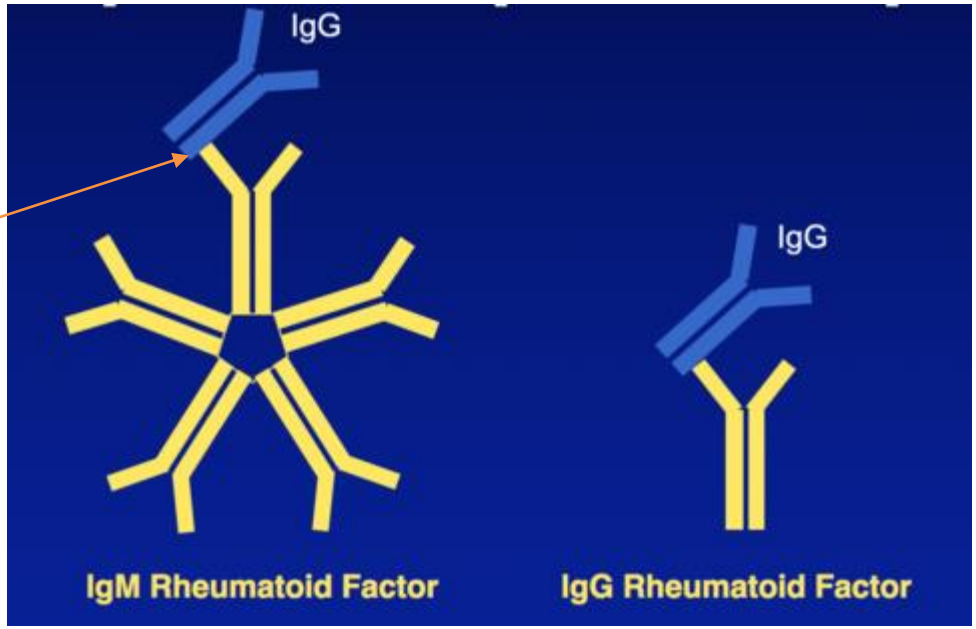


Non-specific reactions

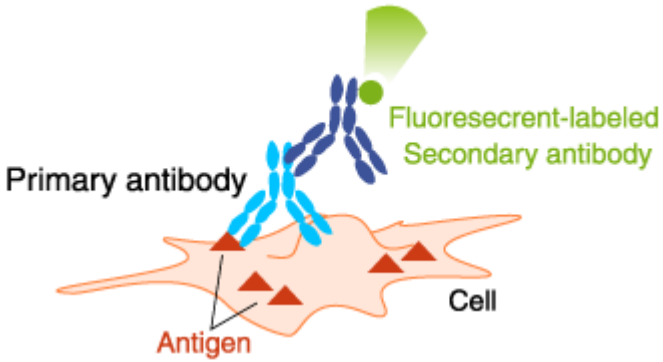


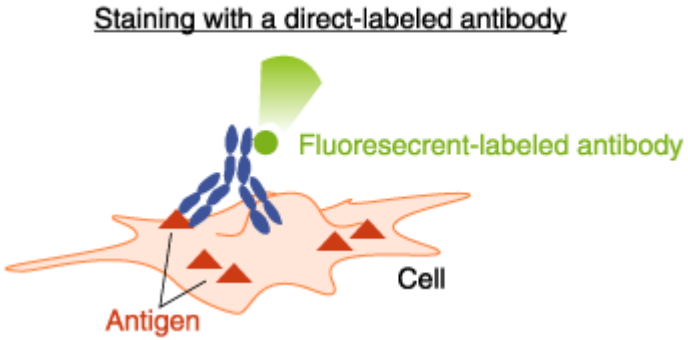
Non-specific reactions

Fc Region

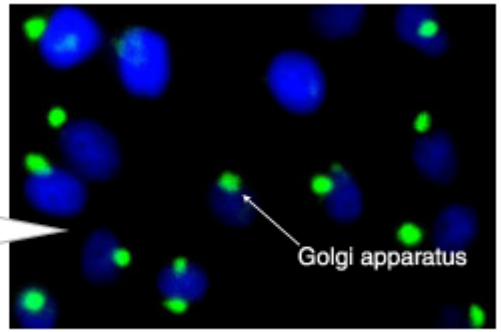


Fluorescence-labeled antibodies: Immunofluorescence



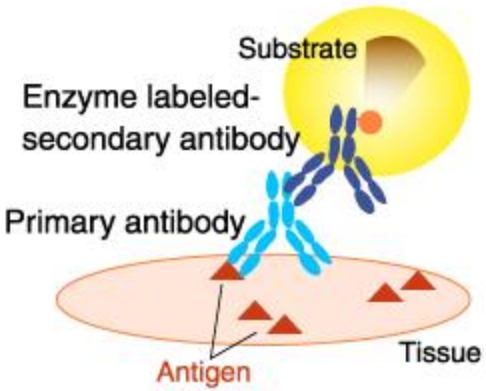


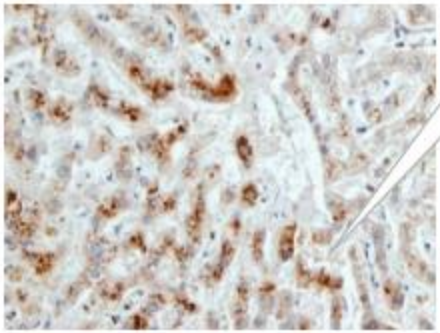
Green fluorescence of the fluoro-phore of the direct-labeled antibody (Alexa Fluor® 488) is observed by a fluorescence microscope.



Sample: HeLa cells

Enzyme-labeled antibodies: Immunohistochemistry



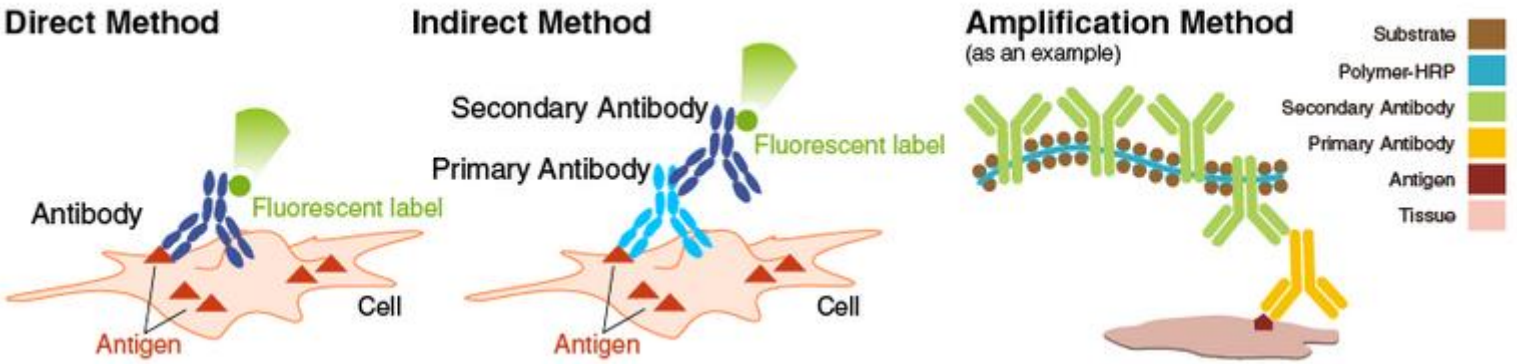


The brown color, generated in the reaction between the substrate DAB and an HRP-labeled secondary antibody, is observed by light microscopy.

Sample: Human liver tissue section

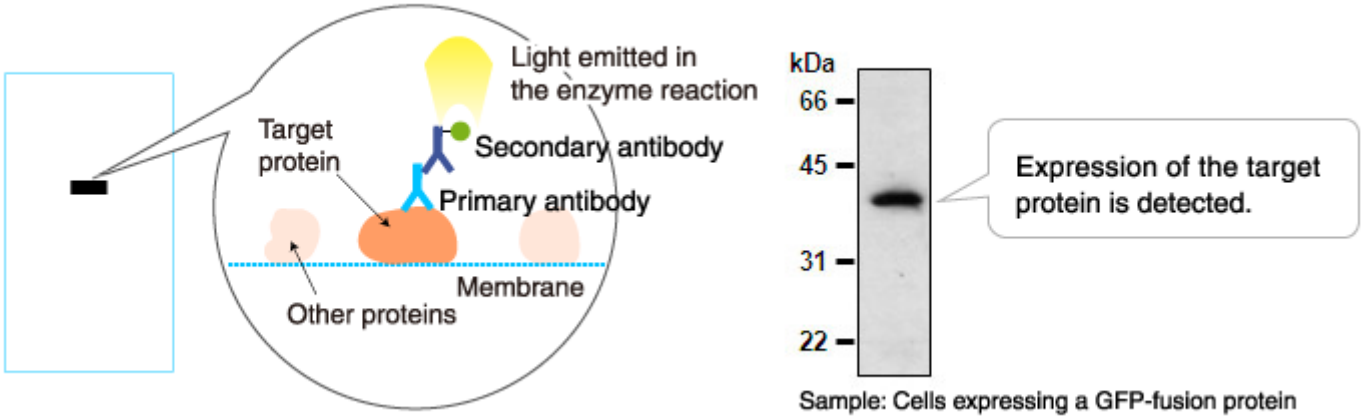
The result of immunohistochemical staining indicates that autophagy was induced in the liver tissue.

Immunohistochemistry

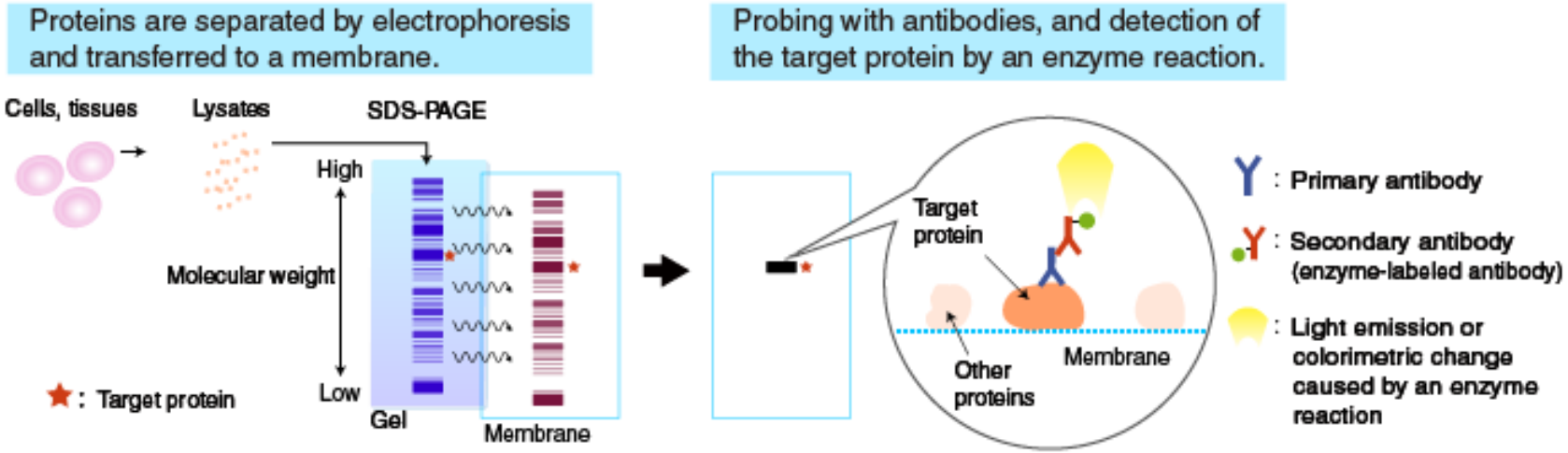


	Advantages	Disadvantages
Direct Method	・Easy for multiple staining, especially Fluorescent Staining method. ・No non-specific staining by secondary antibodies. ・Shorter working time.	・Requires labeled antibodies in the same quantity as the target molecules, which can be costly. ・Commercially available primary antibodies with the desired label may be very limited. ・Some antibodies may lose activity due to labeling.
Indirect Method	・High versatility - the secondary antibodies can be used if they share the same host species with primary antibodies.	・Difficult to detect multiple target molecules simultaneously - unlike the direct method, primary antibodies need to be from different host animals. ・Longer working time required for secondary antibody reaction compared to the direct method. ・Non-specific staining may occur due to secondary antibodies.
Amplification Method	・Very useful for molecules with low expression levels due to the amplification capability.	・Consideration of endogenous biotin presence is required if using the biotin-streptavidin system.

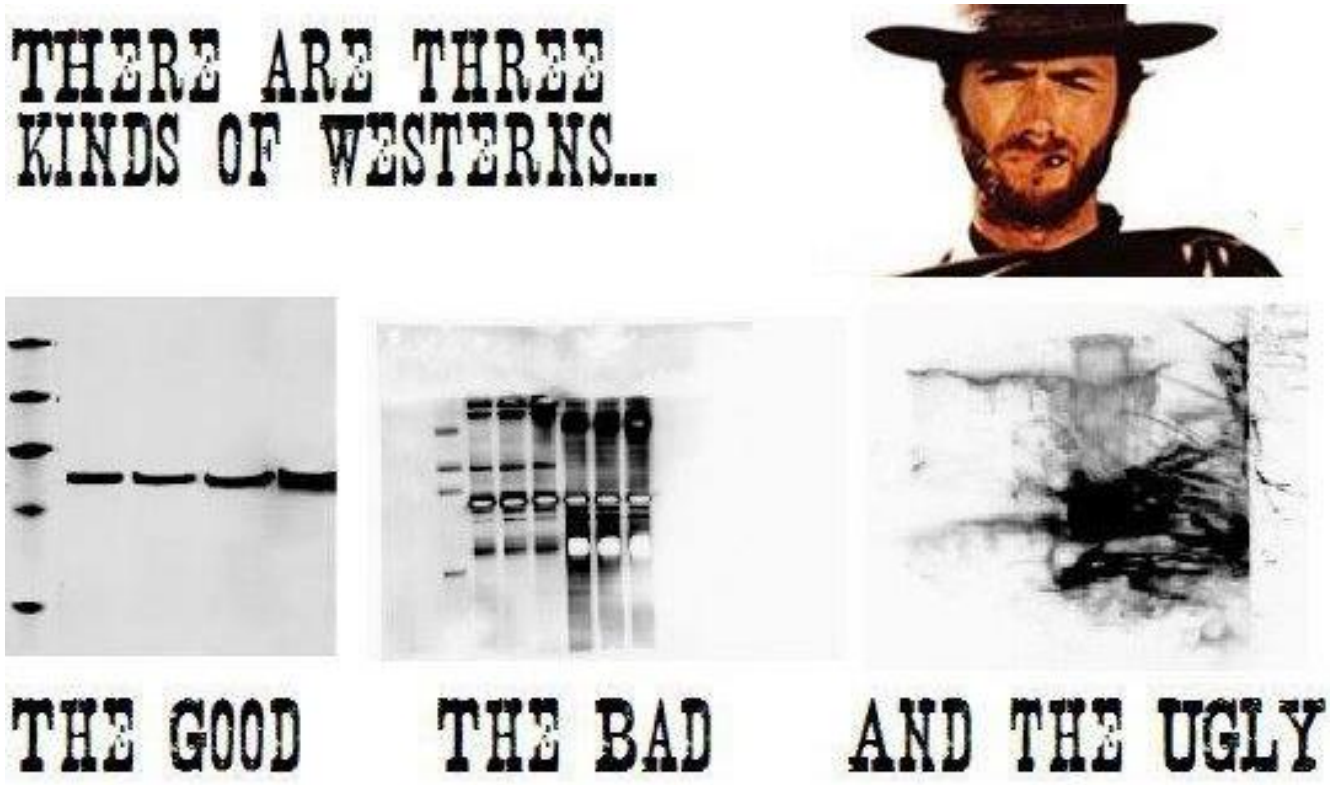
Enzyme-labeled antibodies: Western blotting



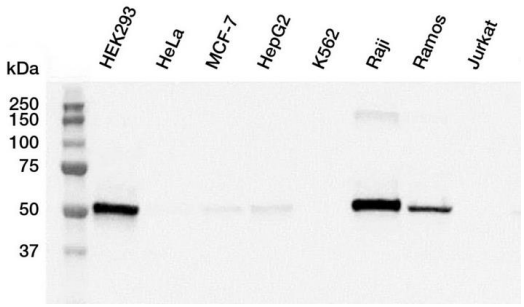
Western blotting



Western blotting



Western blotting



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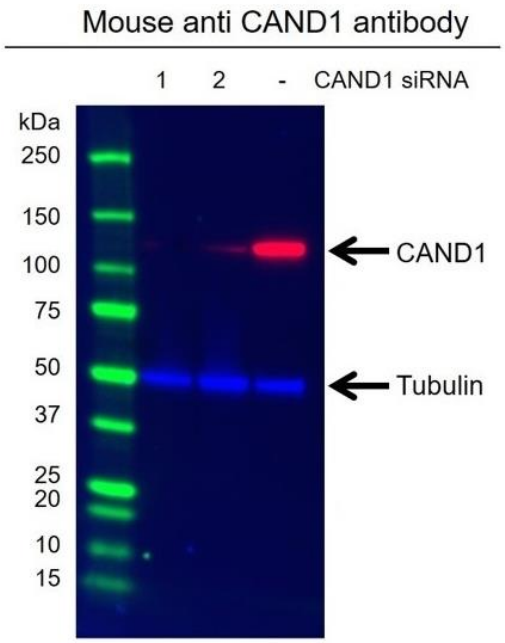
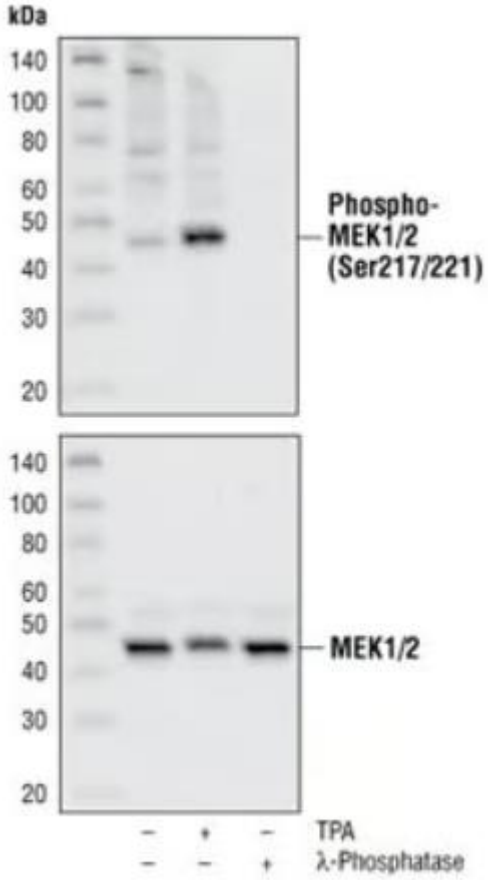
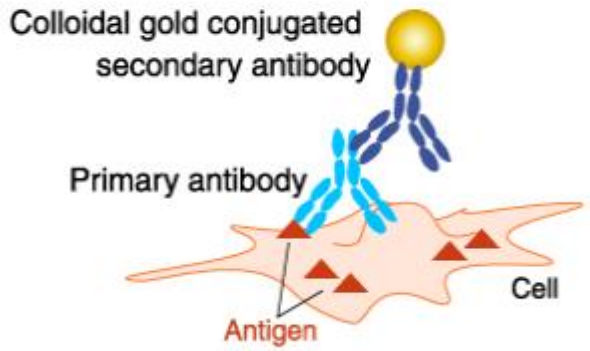
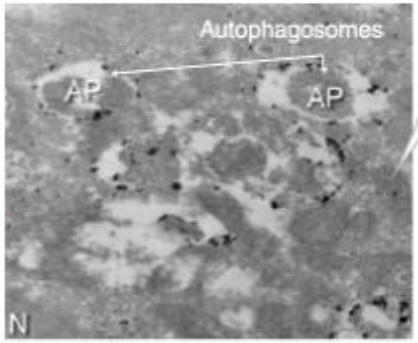




Colloidal gold-labeled antibodies: Microscopy

Immunoelectron micrograph of autophagosomes detected using the marker LC3



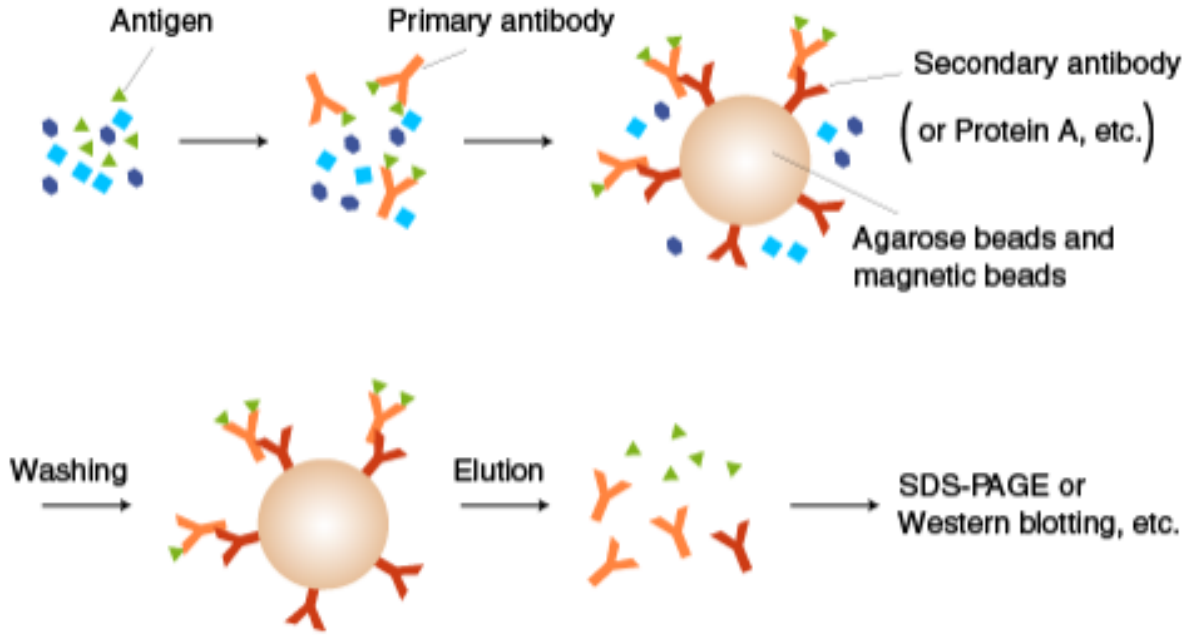


Sample: MEF cells (starved condition)

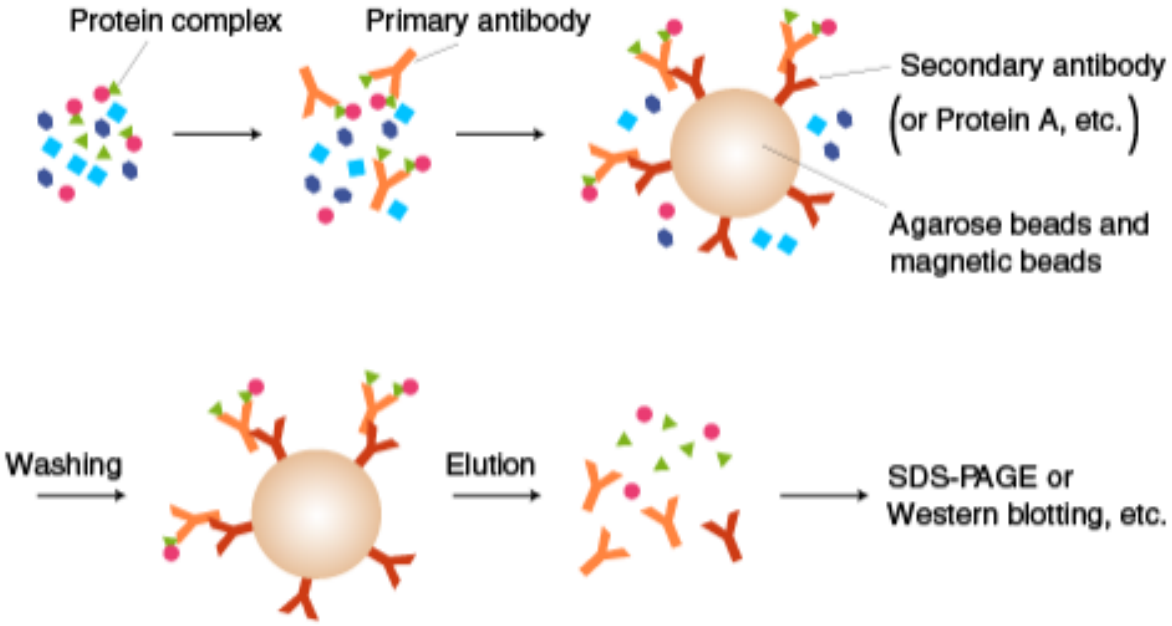
Colloidal gold (black dots), labeled on the secondary antibody, is observed by electron microscopy.

The data were kindly provided by Dr. Noboru Mizushima of the University of Tokyo.

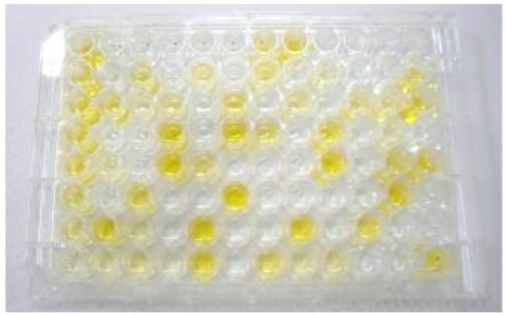
Immunoprecipitation



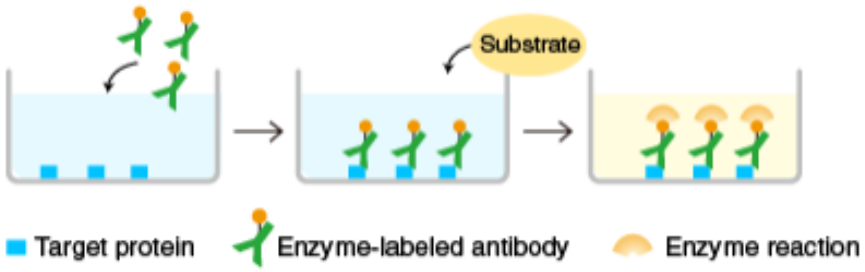
Co-Immunoprecipitation

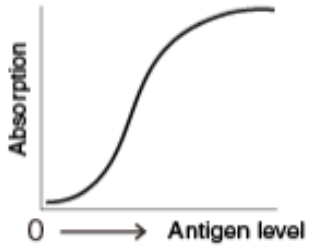


ELISA
(Enzyme-Linked Immunosorbent Assay)



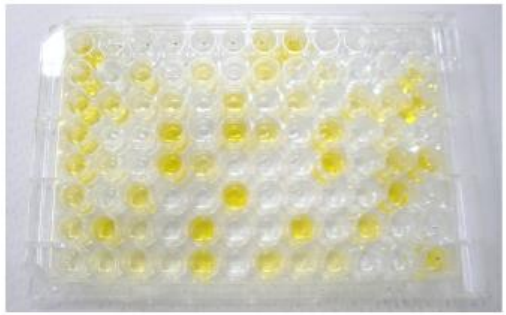
The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.





Direct ELISA

ELISA
(Enzyme-Linked Immunosorbent Assay)



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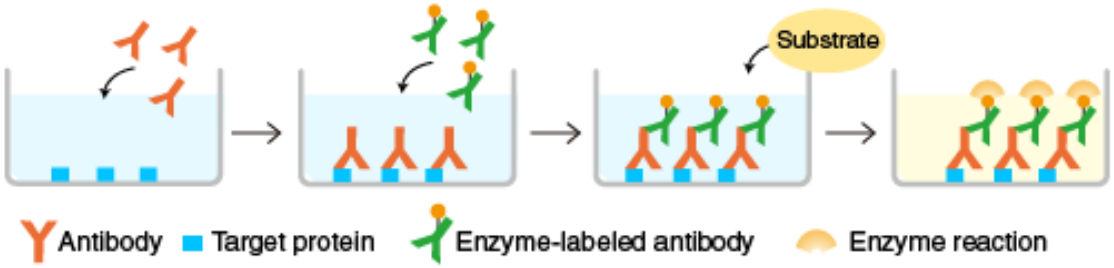
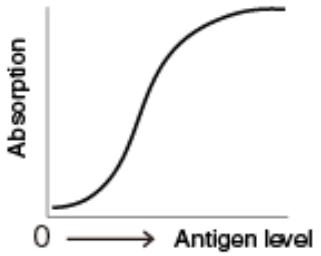
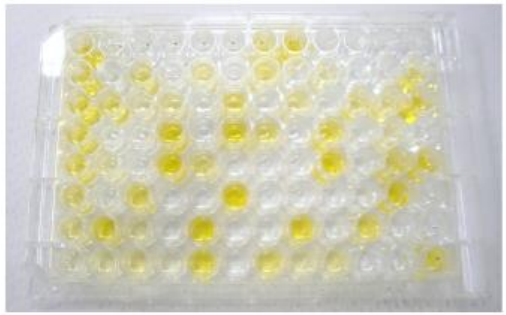


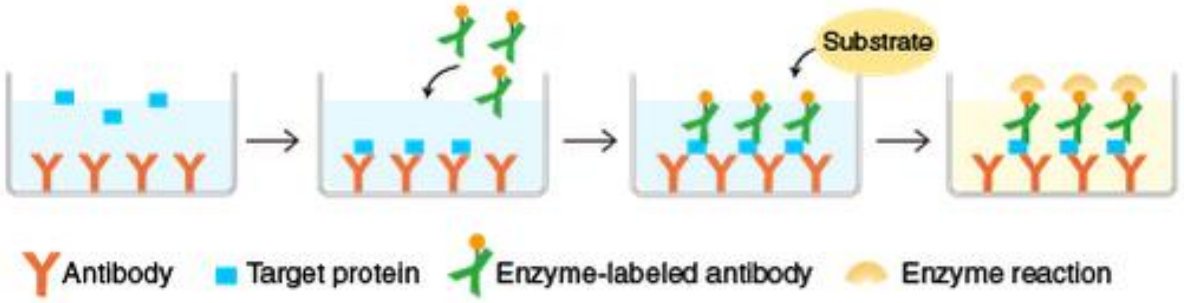


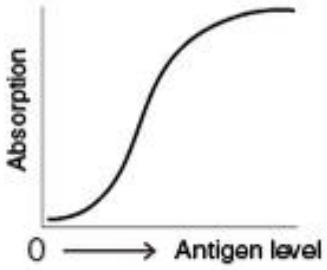
Indirect ELISA

ELISA
(Enzyme-Linked Immunosorbent Assay)



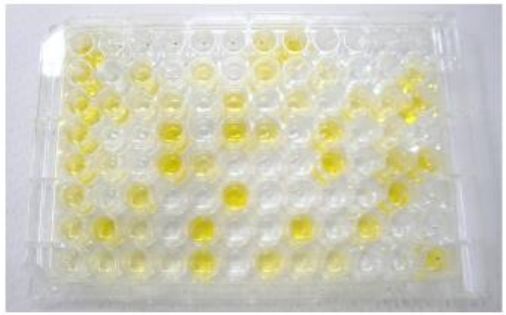
The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.



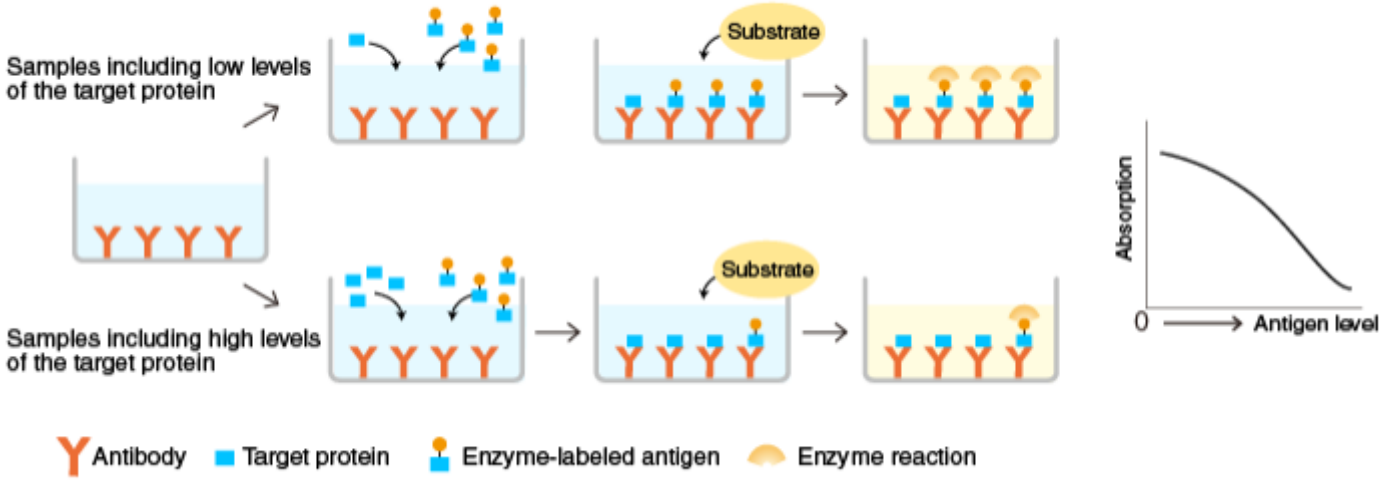


Sandwich ELISA

ELISA
(Enzyme-Linked Immunosorbent Assay)

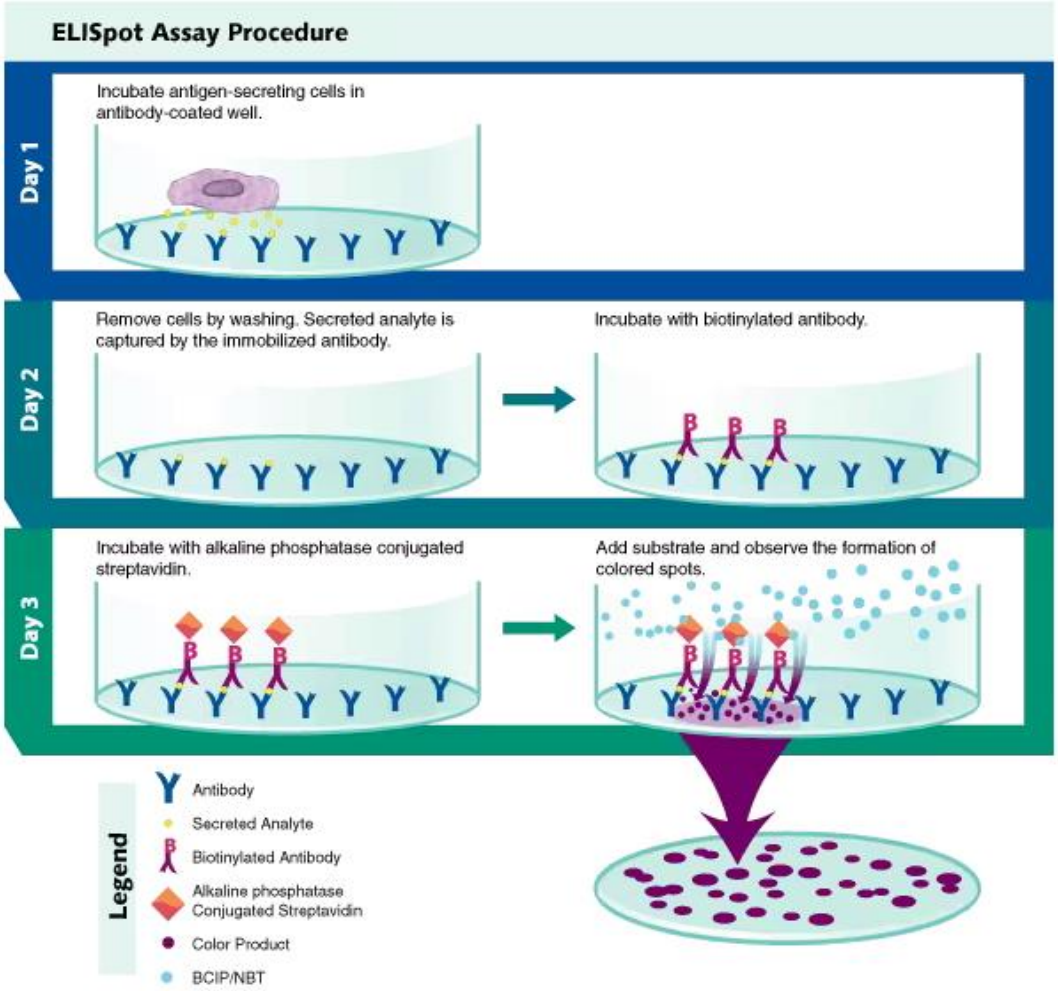


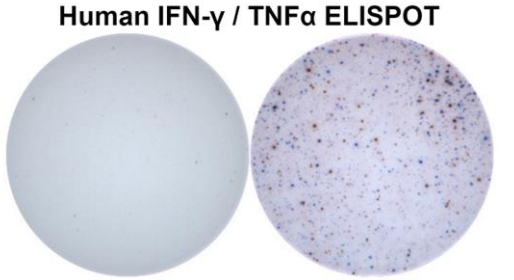
The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.



Competitive ELISA

EliSpot





Human PBMC (25,000 cells/well) incubated overnight with or without PHA stimulation.

Flow cytometry

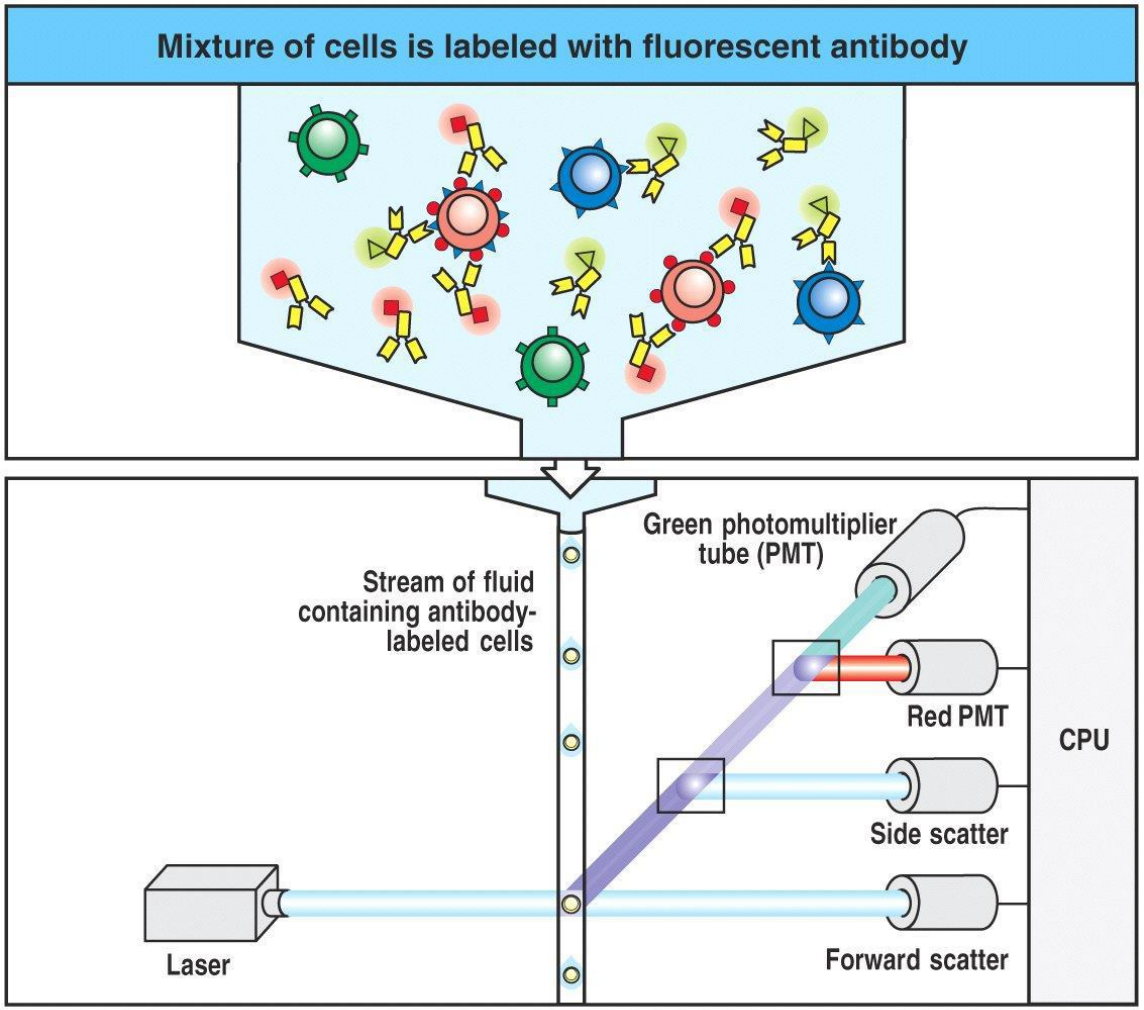
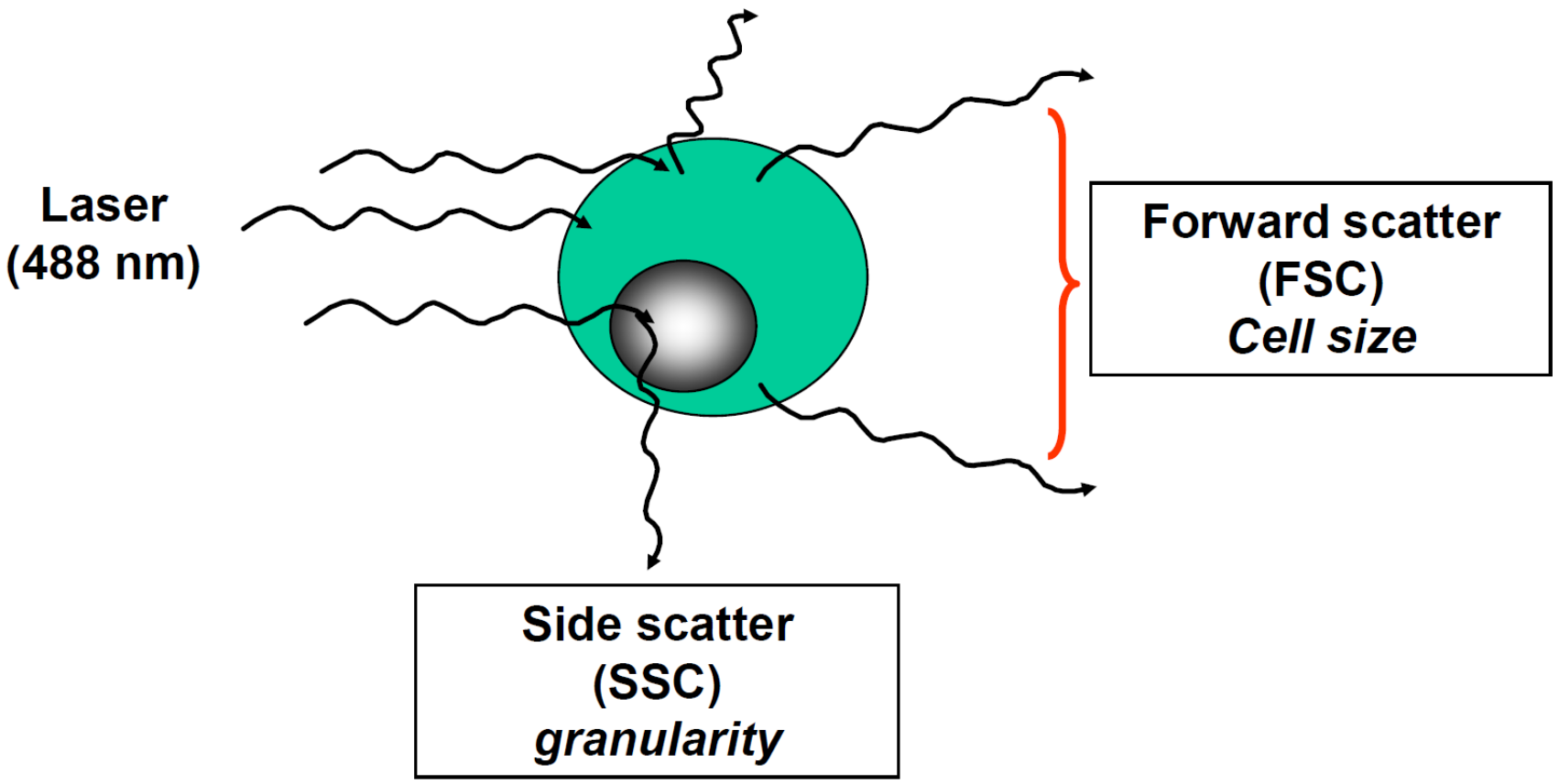
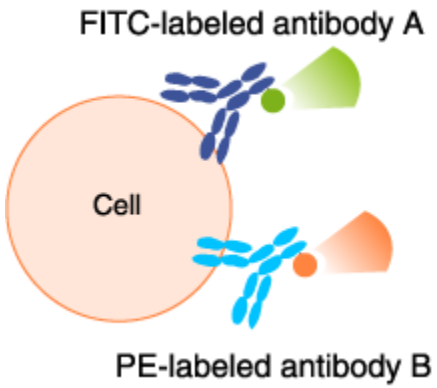


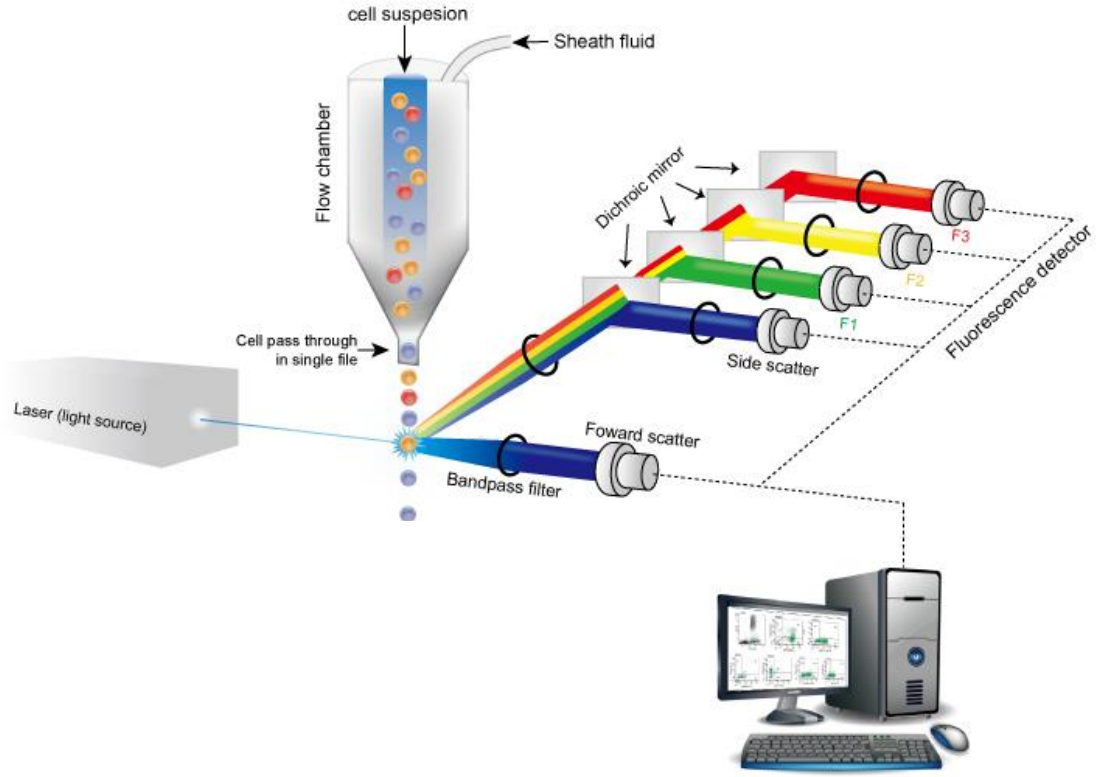
Figure A-25 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Flow cytometry



Flow cytometry





Flow cytometry

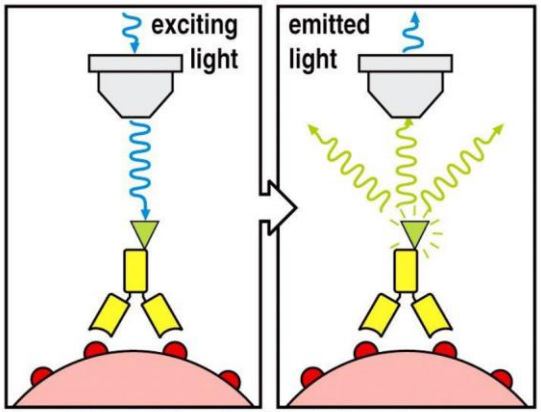
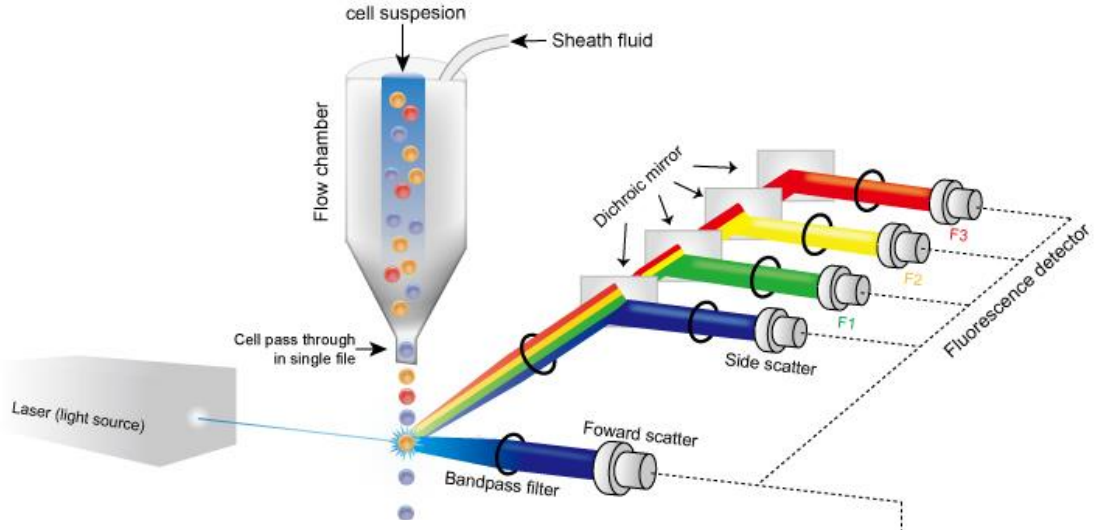
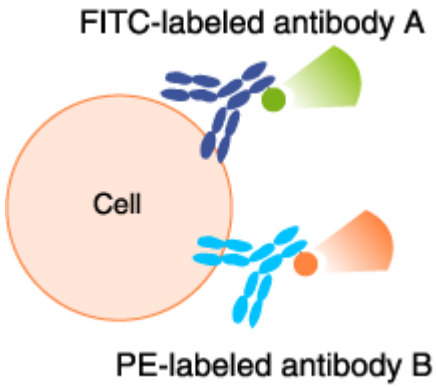
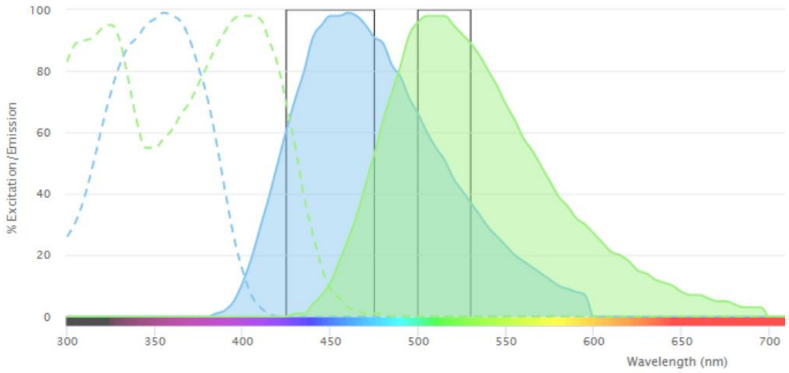


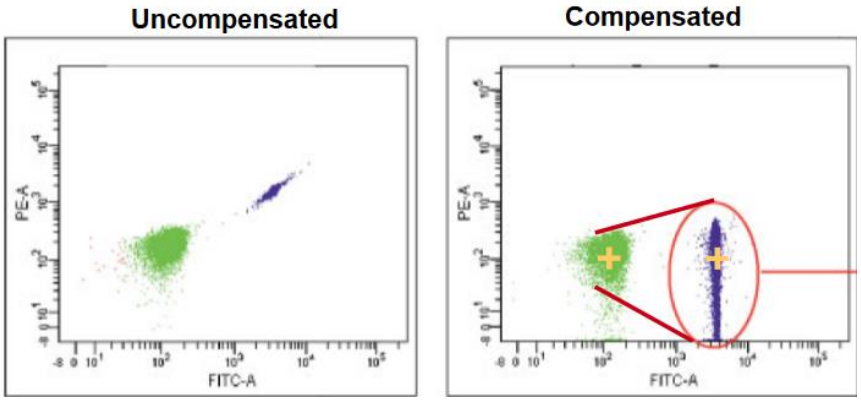
Figure A-17 Immunobiology, 6/e. (© Garland Science 2005)

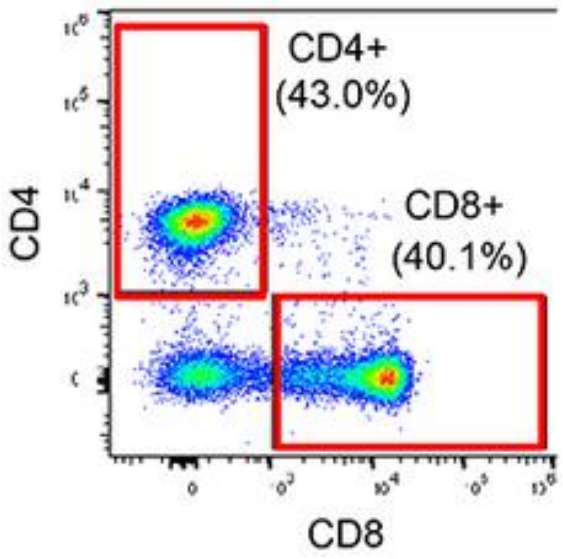


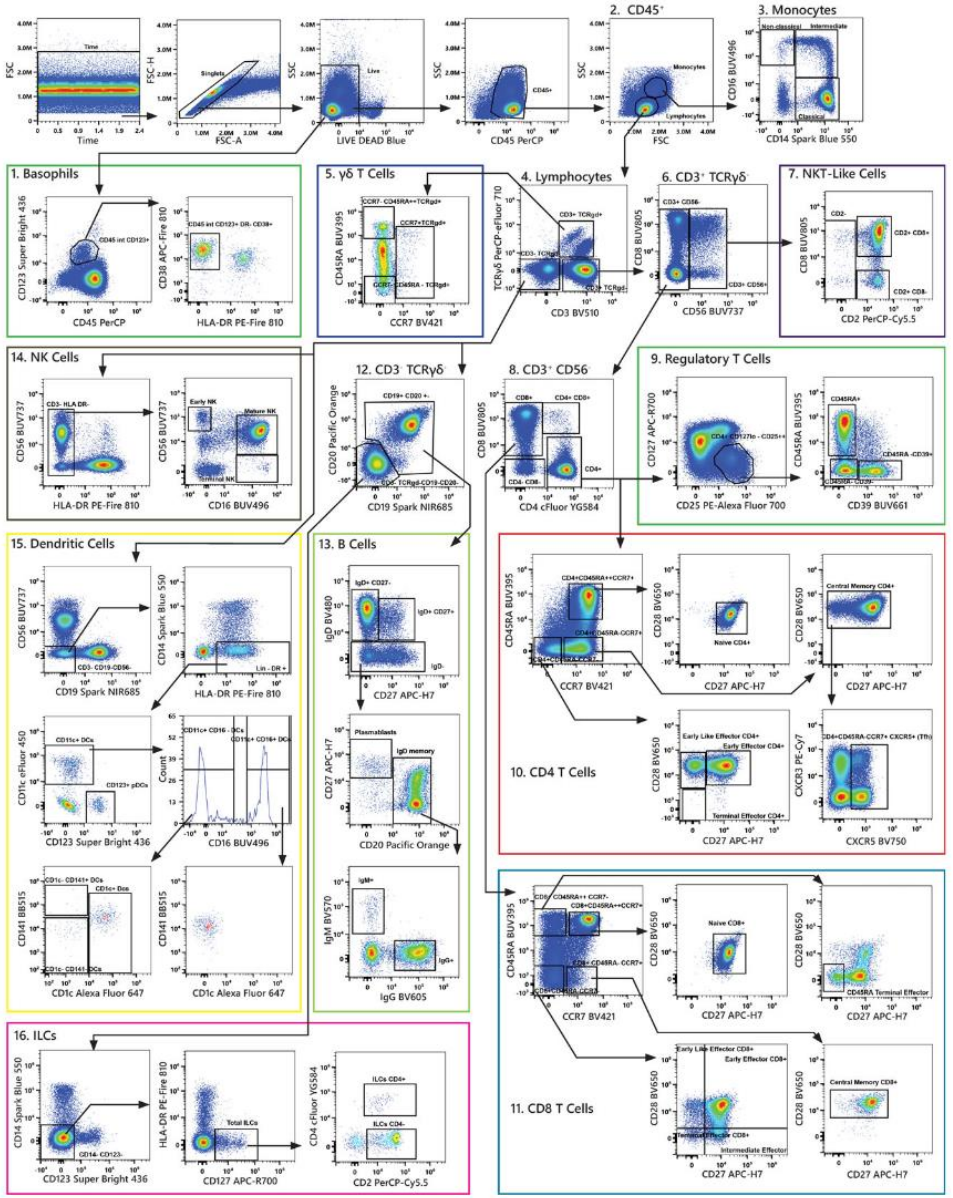


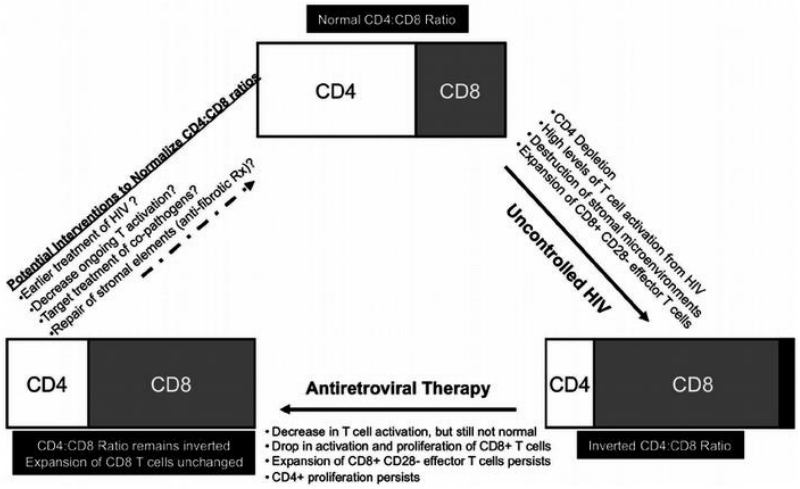
Flow cytometry






CD4+
(43.0%)
CD8+
(40.1%)
CD4
CD8


2. CD45+
3. Monocytes
1. Basophils
5. γδ T Cells
4. Lymphocytes
6. CD3+ TCRγδ-
7. NKT-Like Cells
14. NK Cells
12. CD3- TCRγδ-
8. CD3+ CD56-
9. Regulatory T Cells
15. Dendritic Cells
13. B Cells
10. CD4 T Cells
16. ILCs
11. CD8 T Cells



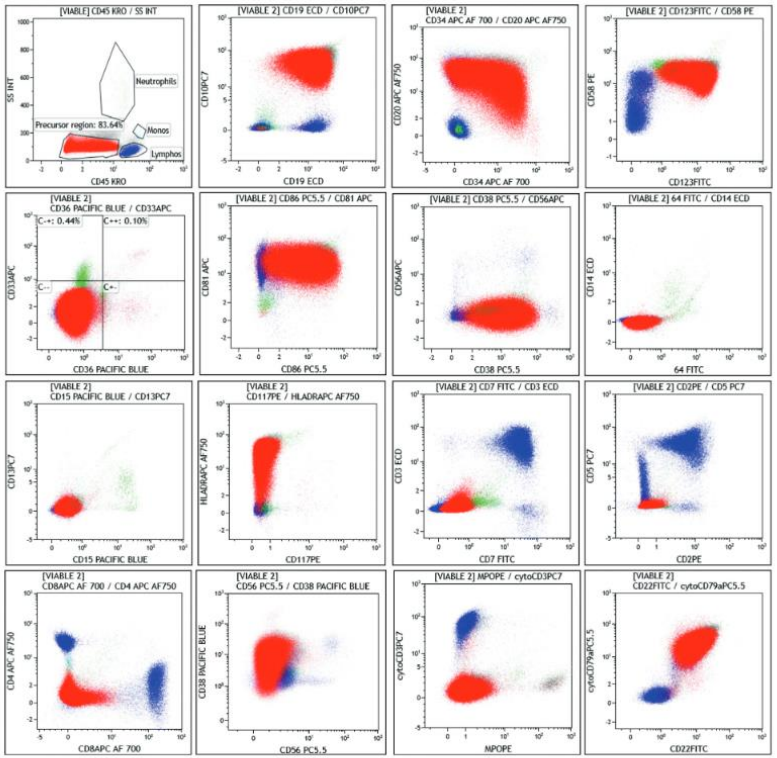
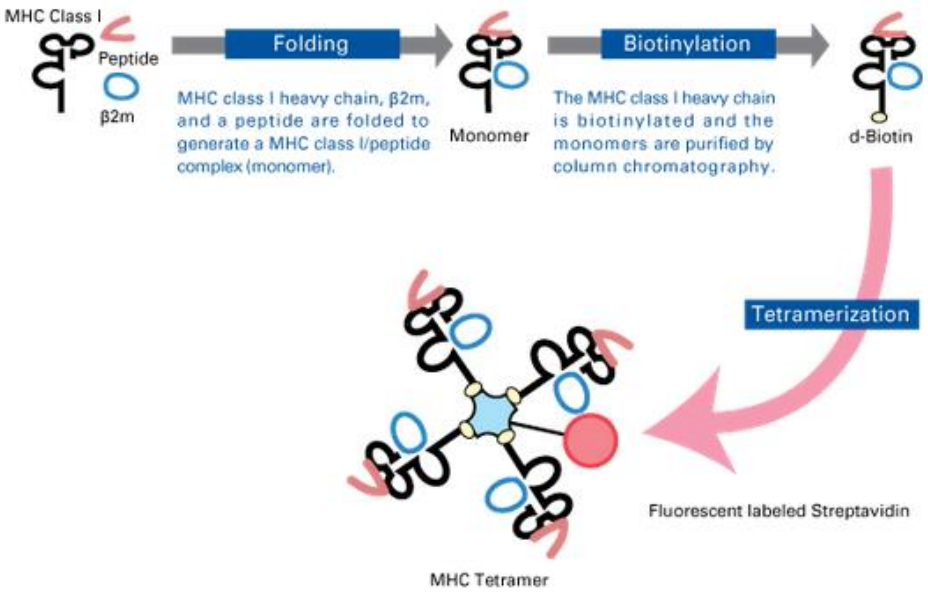
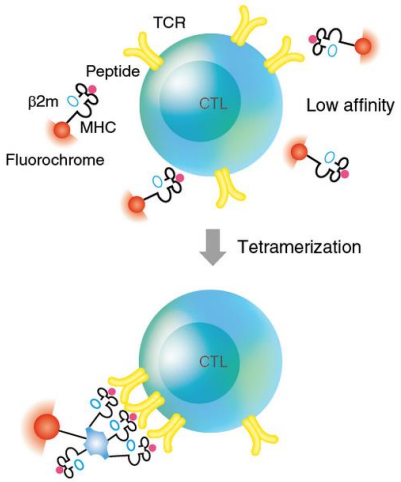
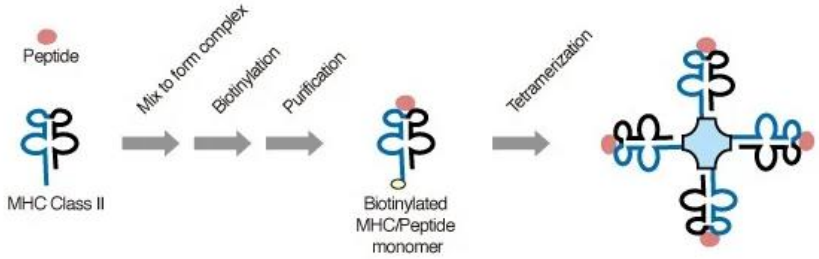


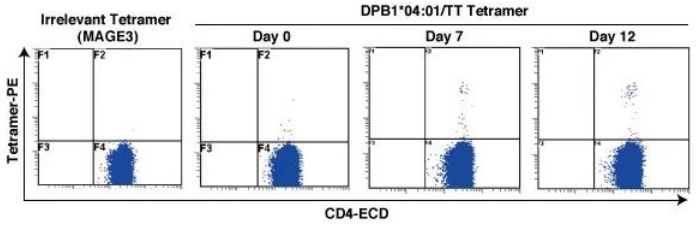
Fig. 1 Flow cytometric dot plots of a case of BCP-ALL. The blasts (*red population*) are SSC low, CD45 dim to negative, positive for CD19, CD10, CD34, CD20, CD58, CD123, CD81, CD86, CD38, HLA-DR, CD22, and CytoCD79a. T-cell markers like CD3, CD7, CD5, CD2, CD4, CD8 and NK cell marker like CD56, and, myeloid markers like CD13, CD15, CD33, CD26, CD117, CD14, CD64 and MPO are negative.

MHC Tetramers

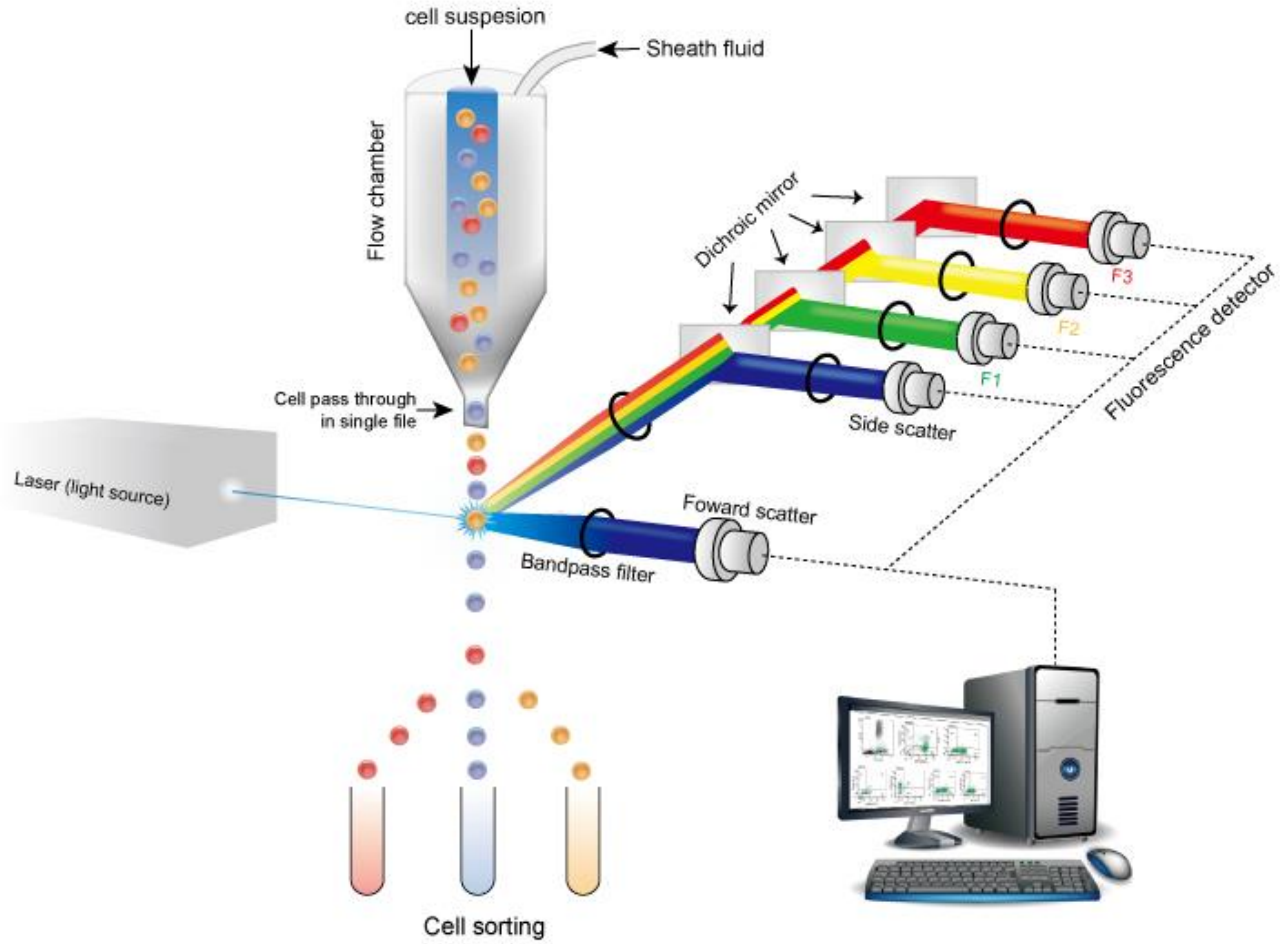




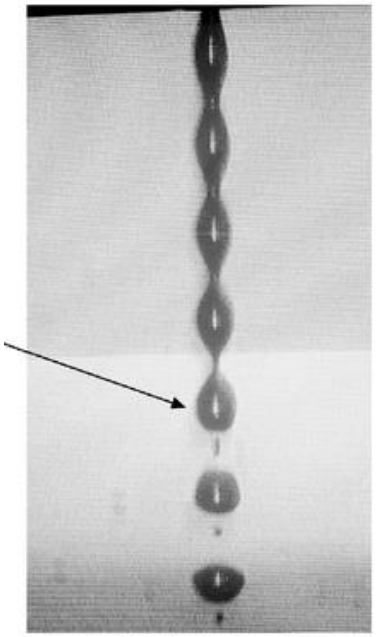


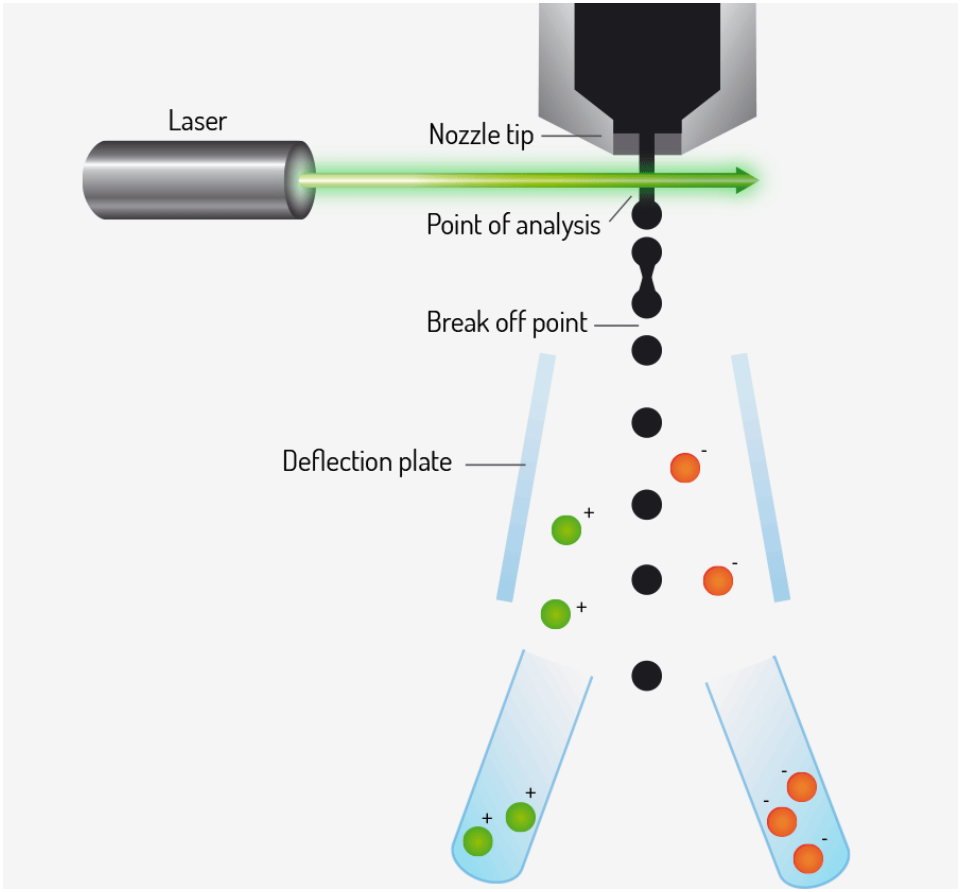


Cell Sorting



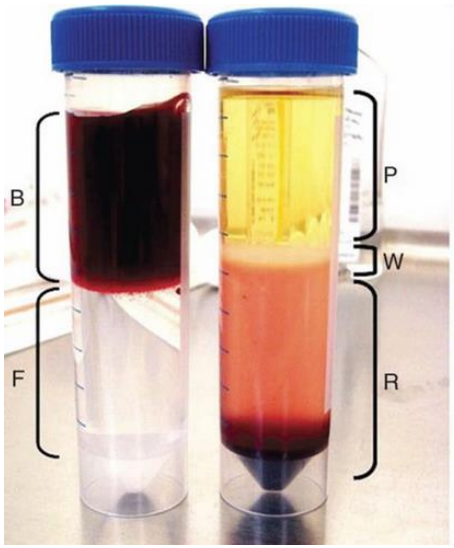
Cell Sorting

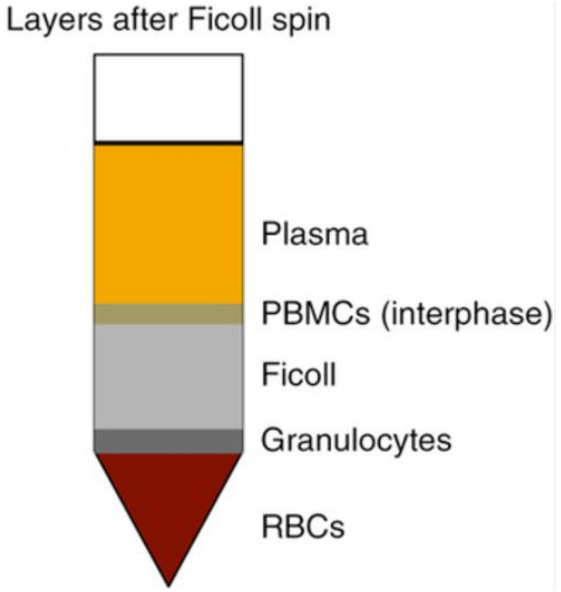




Cell isolation:
Ficoll-paque gradient centrifugation

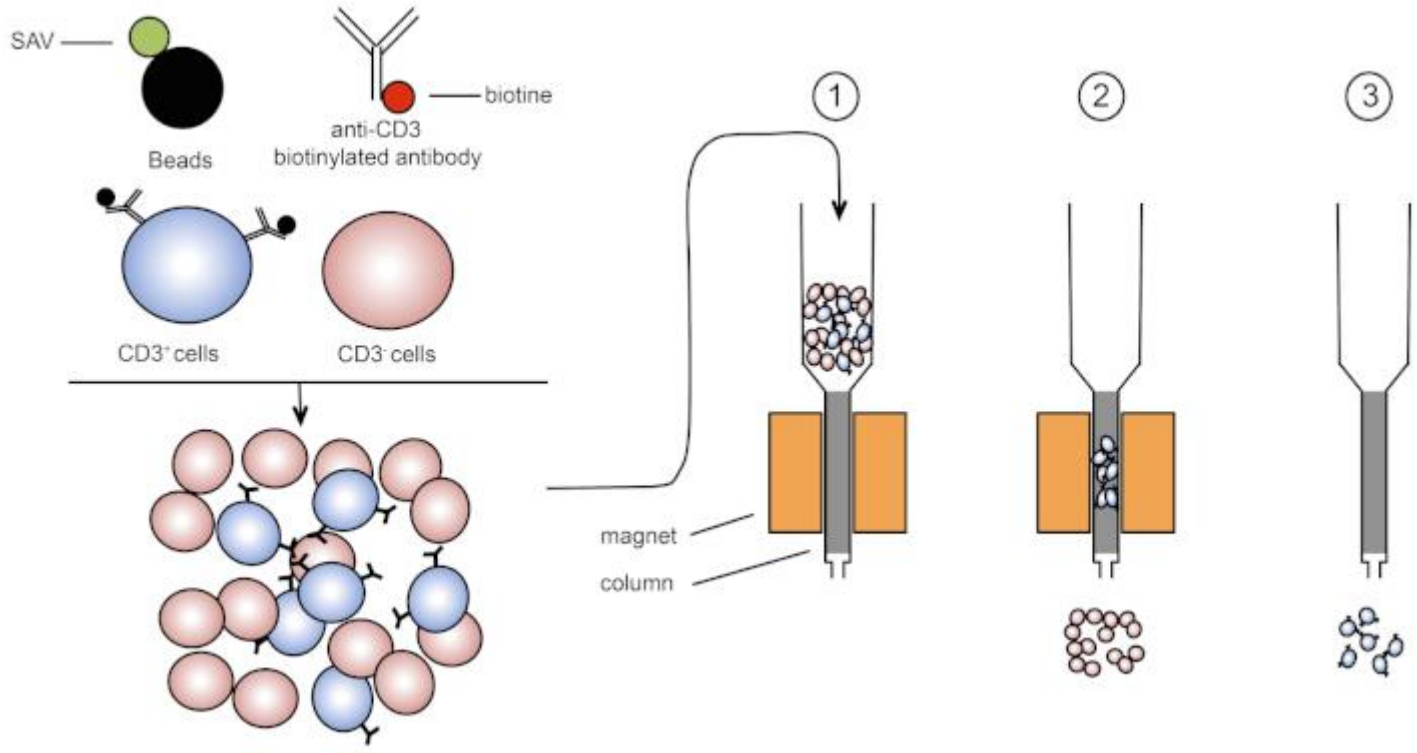
Ficoll:
hydrophylic polysaccharide





Separation of Peripheral Blood Mononuclear Cells (PBMCs) based on density after centrifugation

Cell isolation:
Magnetic separation



Blood group

Serum from individuals of type	Red blood cells from individuals of type: O	A	B	AB
	Express the carbohydrate structures: R–GlcNAc–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc)	R–GlcNAc–Gal–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc) + R–GlcNAc–Gal–Gal (Fuc)
O Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
A Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
B Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
AB No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

Determination of blood group

Blood

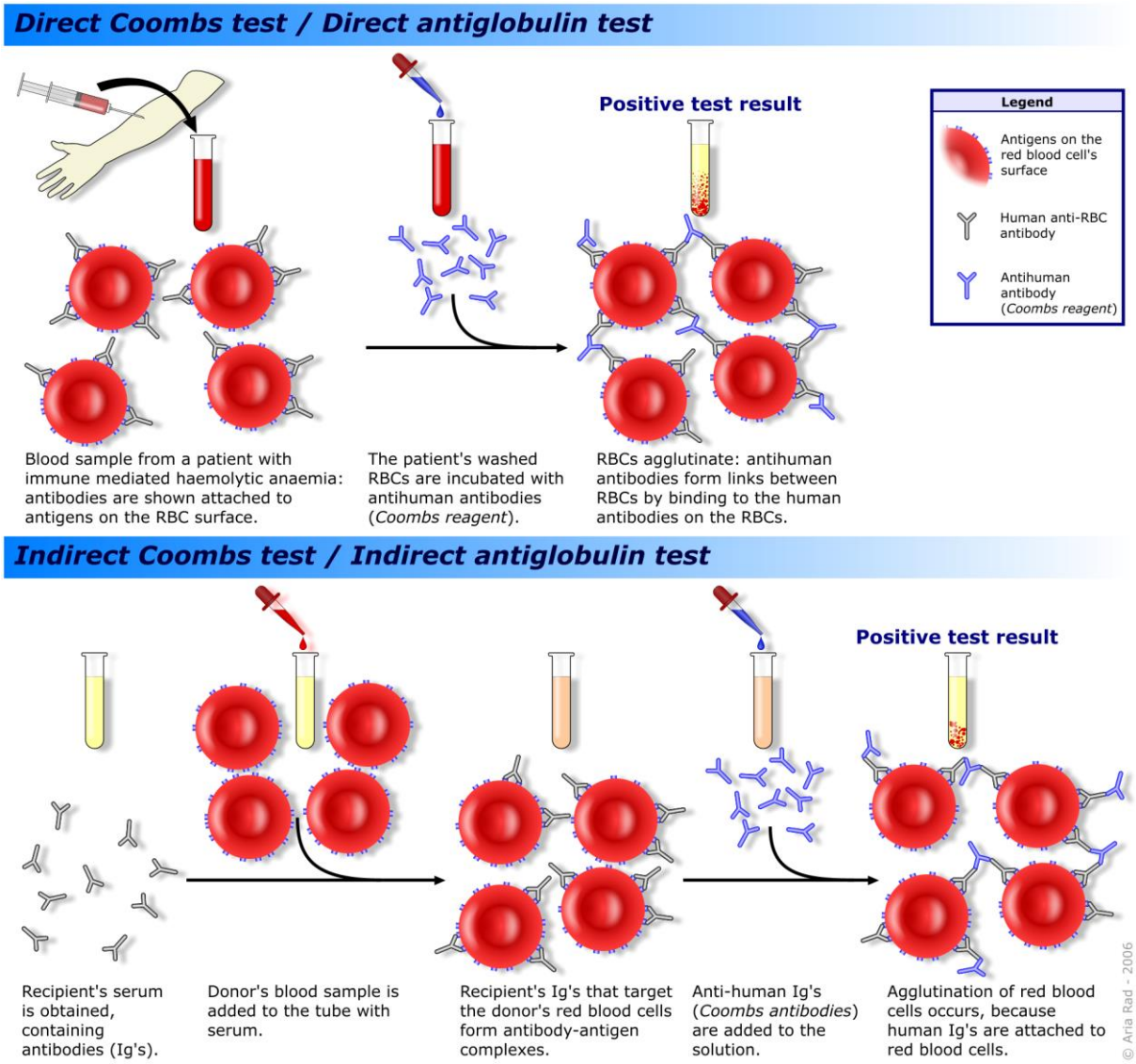
Serum containing:

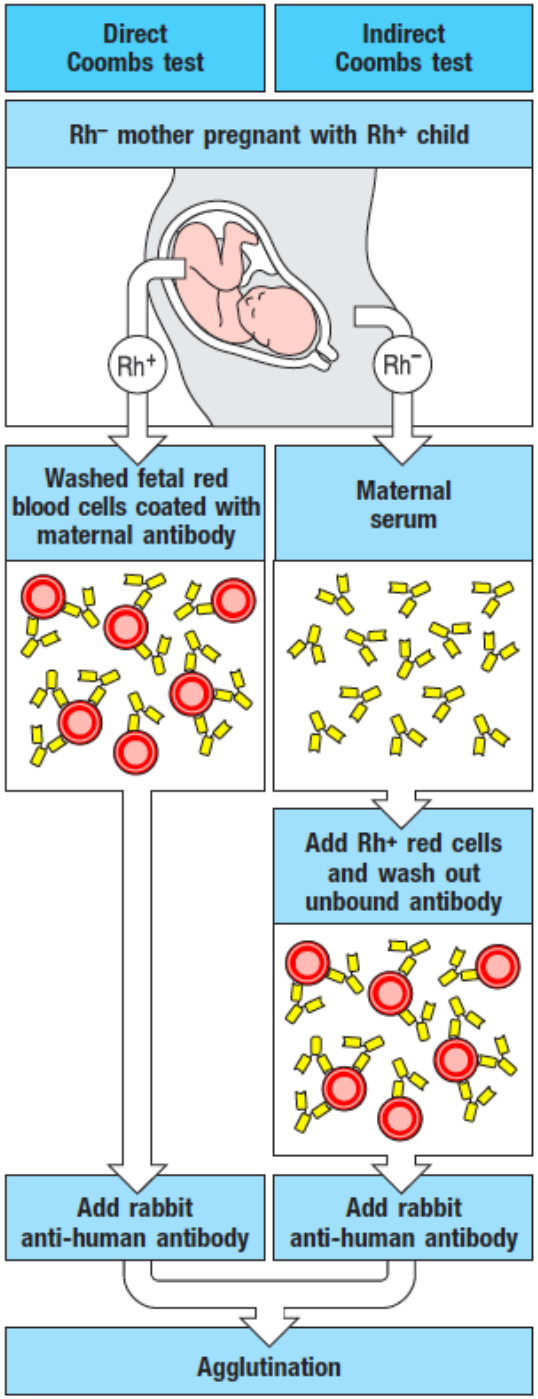
	A	B	AB	O	Rh+	Rh-
Anti-A						
Anti-B						
Anti-A y Anti- B						
Anti-Rh						

Agglutination

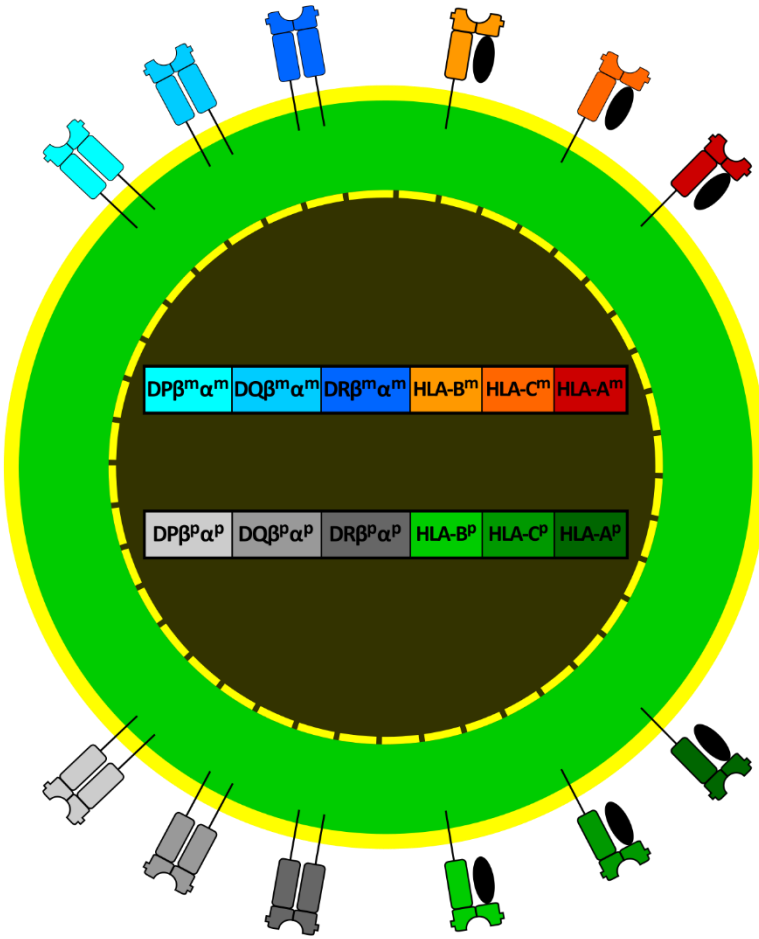
No agglutination

Determination of the donor/recipient compatibility for blood transfusion by agglutination of Erythrocytes with a specific antiserum: Indirect Coombs-Test.

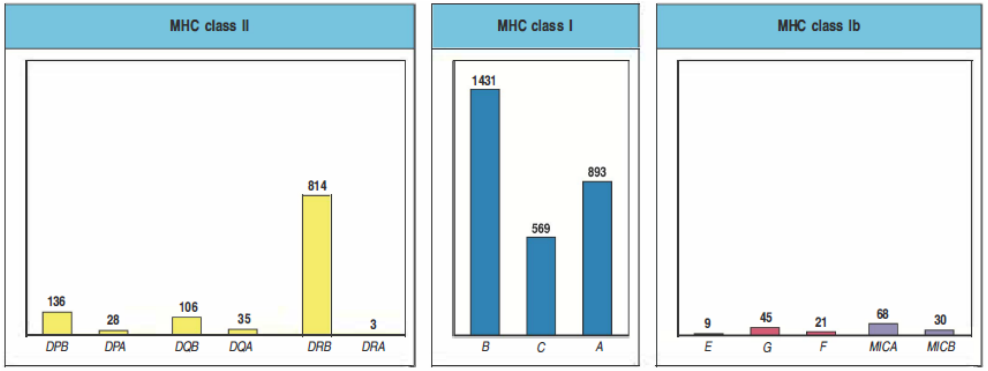


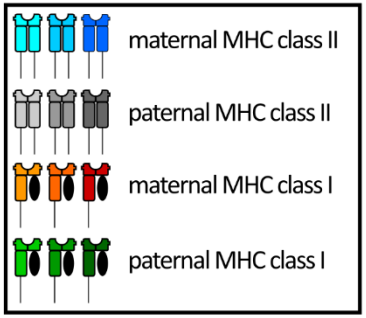

Direct Coombs test
Indirect Coombs test
Rh⁻ mother pregnant with Rh+ child
Rh+
Rh⁻
Washed fetal red blood cells coated with maternal antibody
Maternal serum
Add Rh+ red cells and wash out unbound antibody
Add rabbit anti-human antibody
Add rabbit anti-human antibody
Agglutination

HLA typing by serology

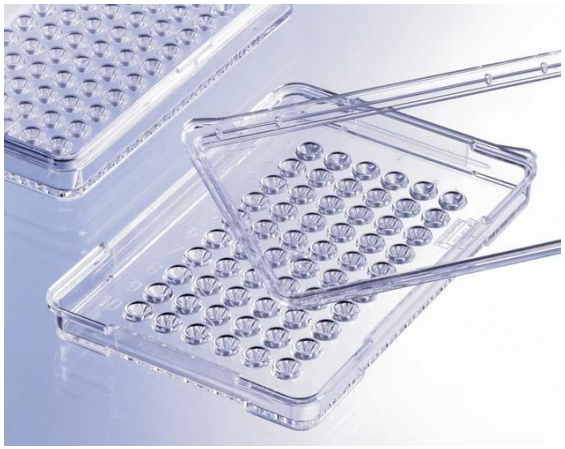


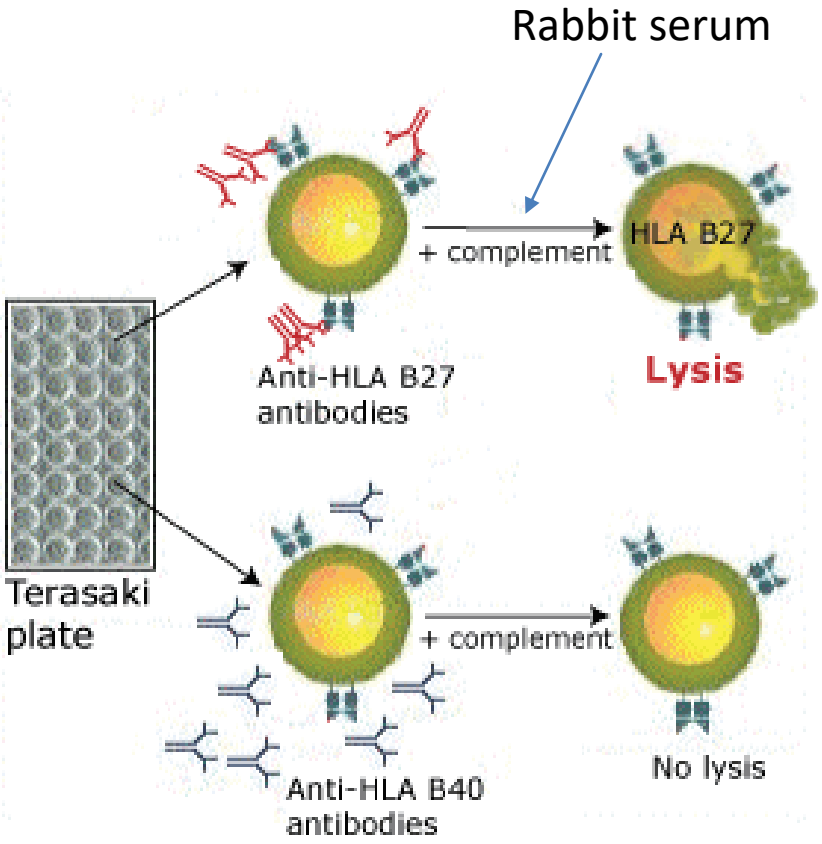
High polymorphic genes





HLA typing by serology



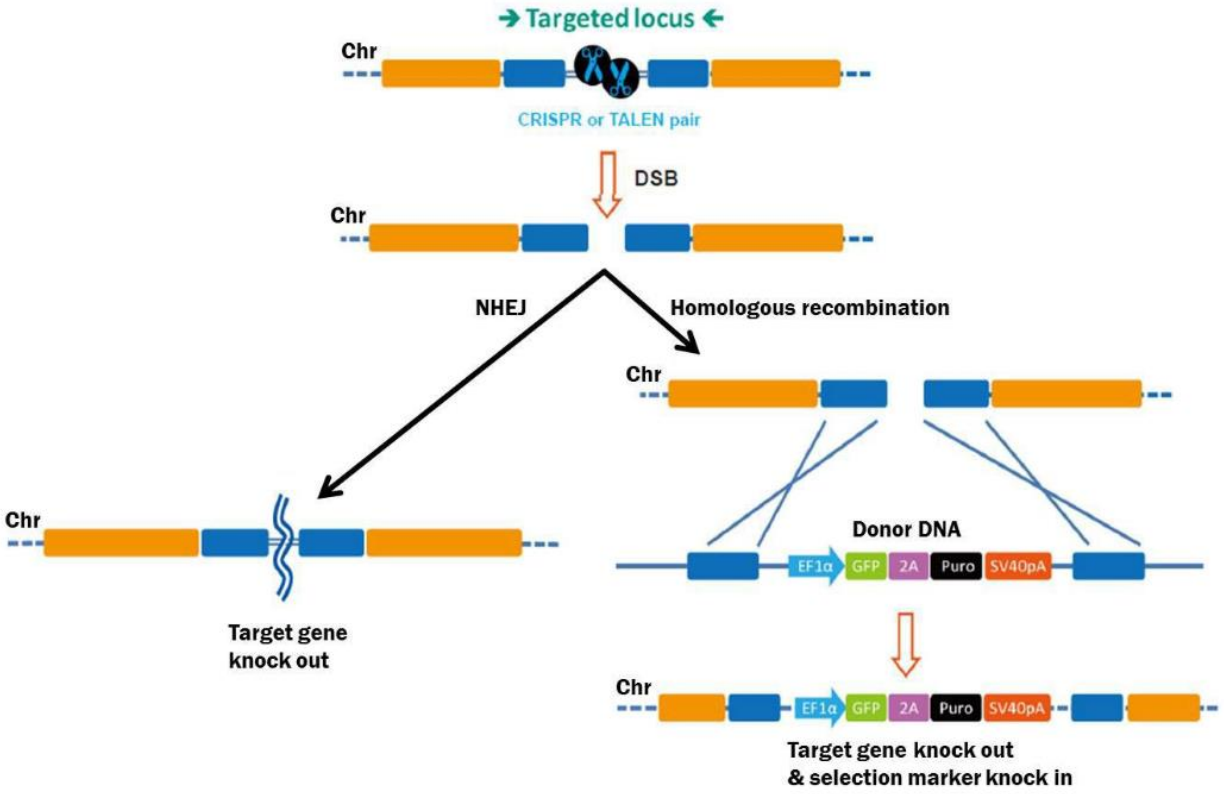



Thermo


Welcome
HiSeq 4000
illumina

Genomic edition

Knockouts (KO) and knockins (KI)

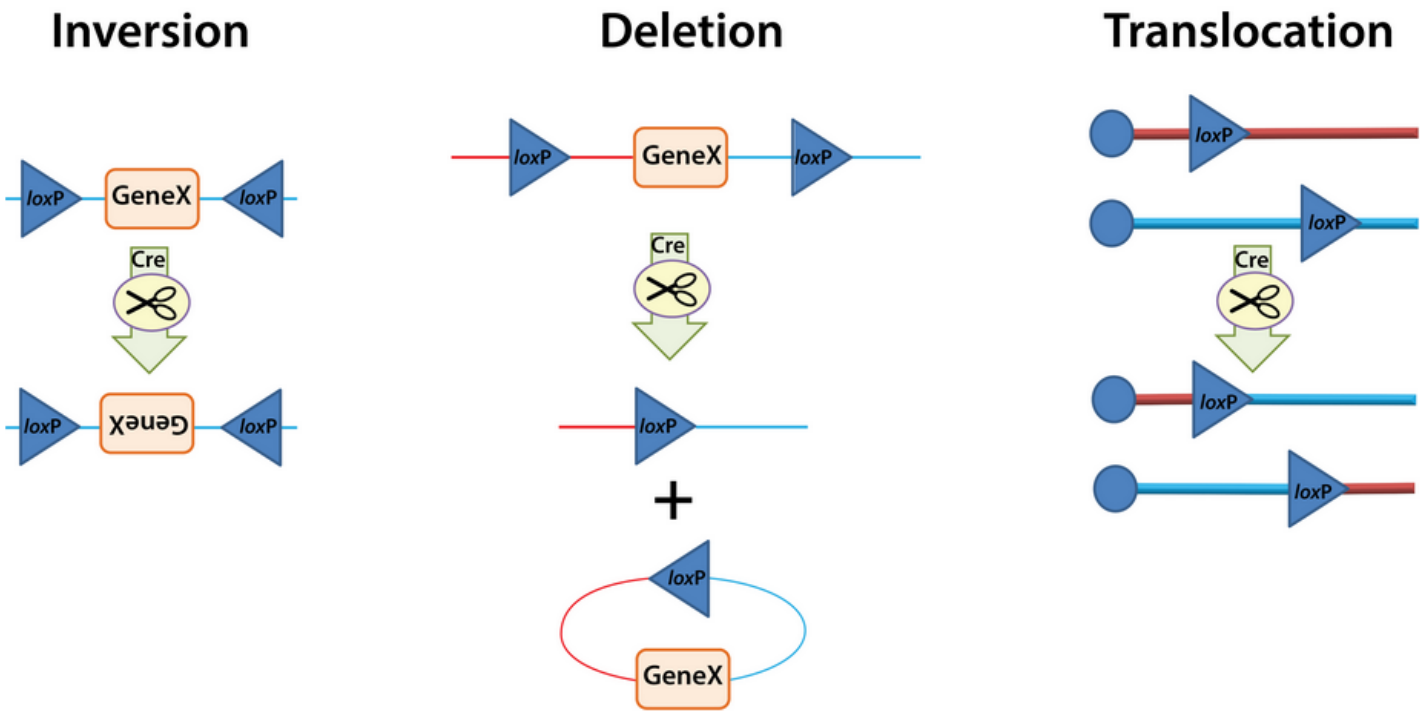


Genomic edition

Conditional or inducible systems

Cre-Lox system:components derived from the P1 bacteriophage:

- 34-base-pair long recognition sequences (loxP)
- Cre recombinase



Immunotherapy

Immunotherapy

Immune System Modulators

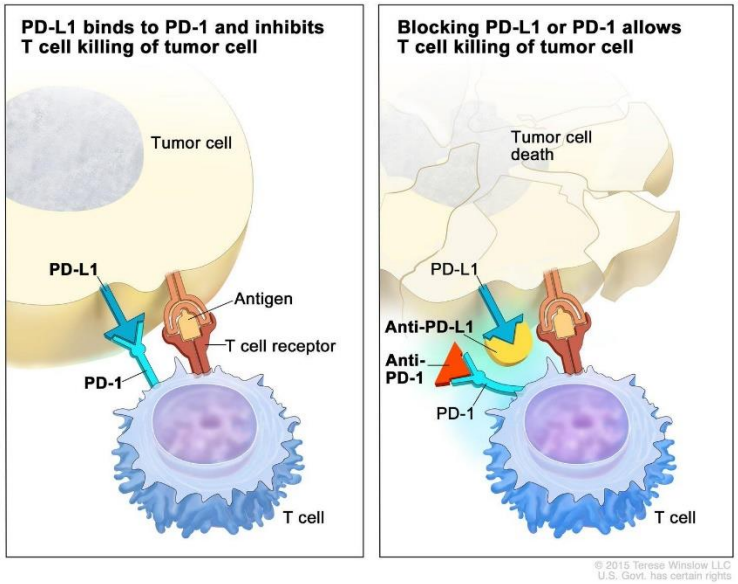
Cytokines: IFNα, Interleukins

Immunomodulatory drugs

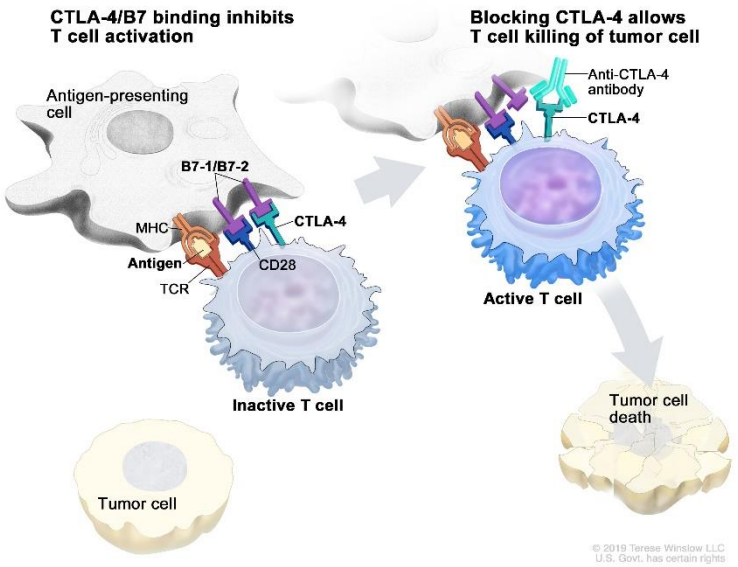
Monoclonal antibodies

Immunotherapy

Immuncheck inhibitors



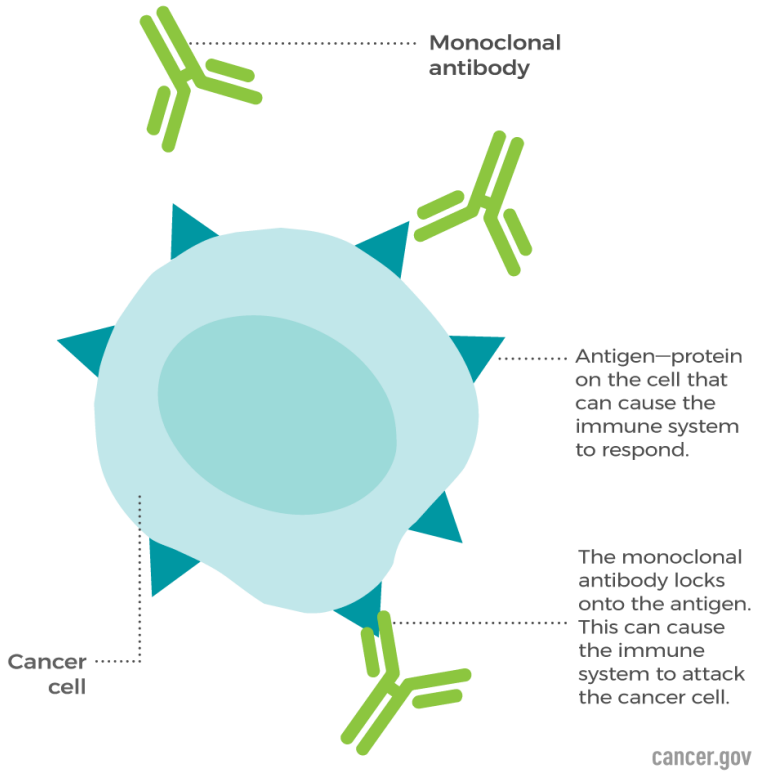
PD-1



CTLA-4

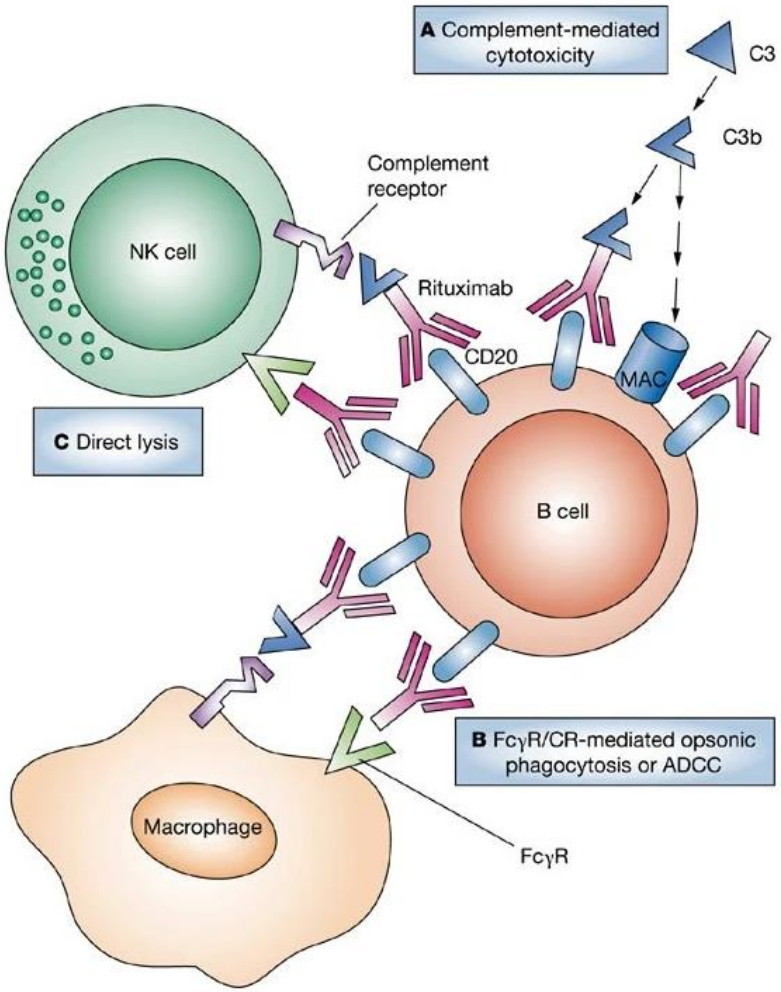
Immunotherapy

Monoclonal antibodies



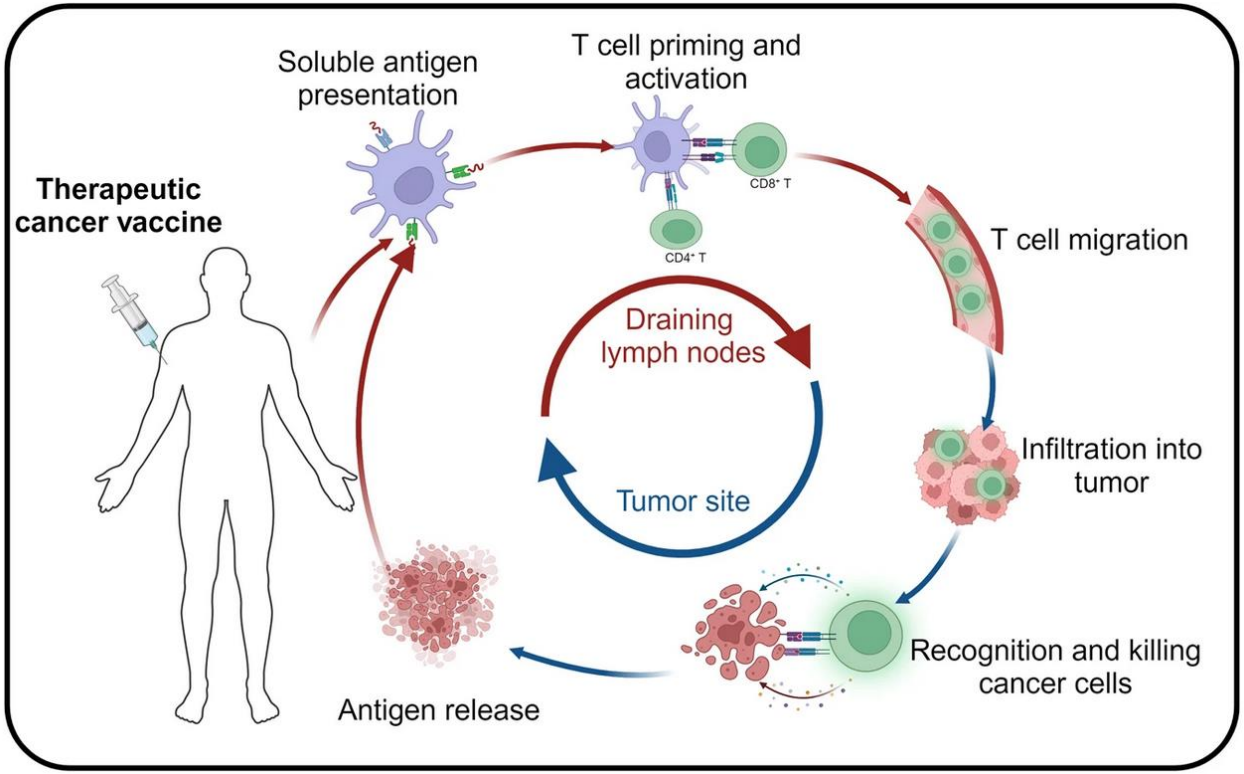
Immunotherapy

Monoclonal antibodies

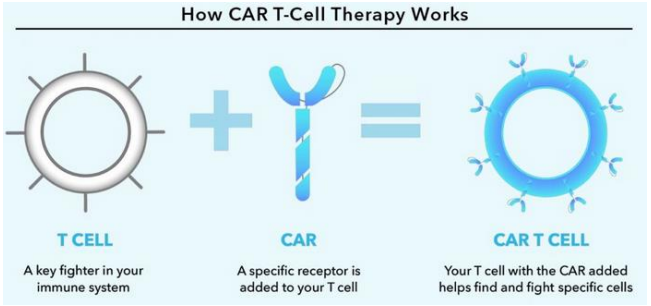


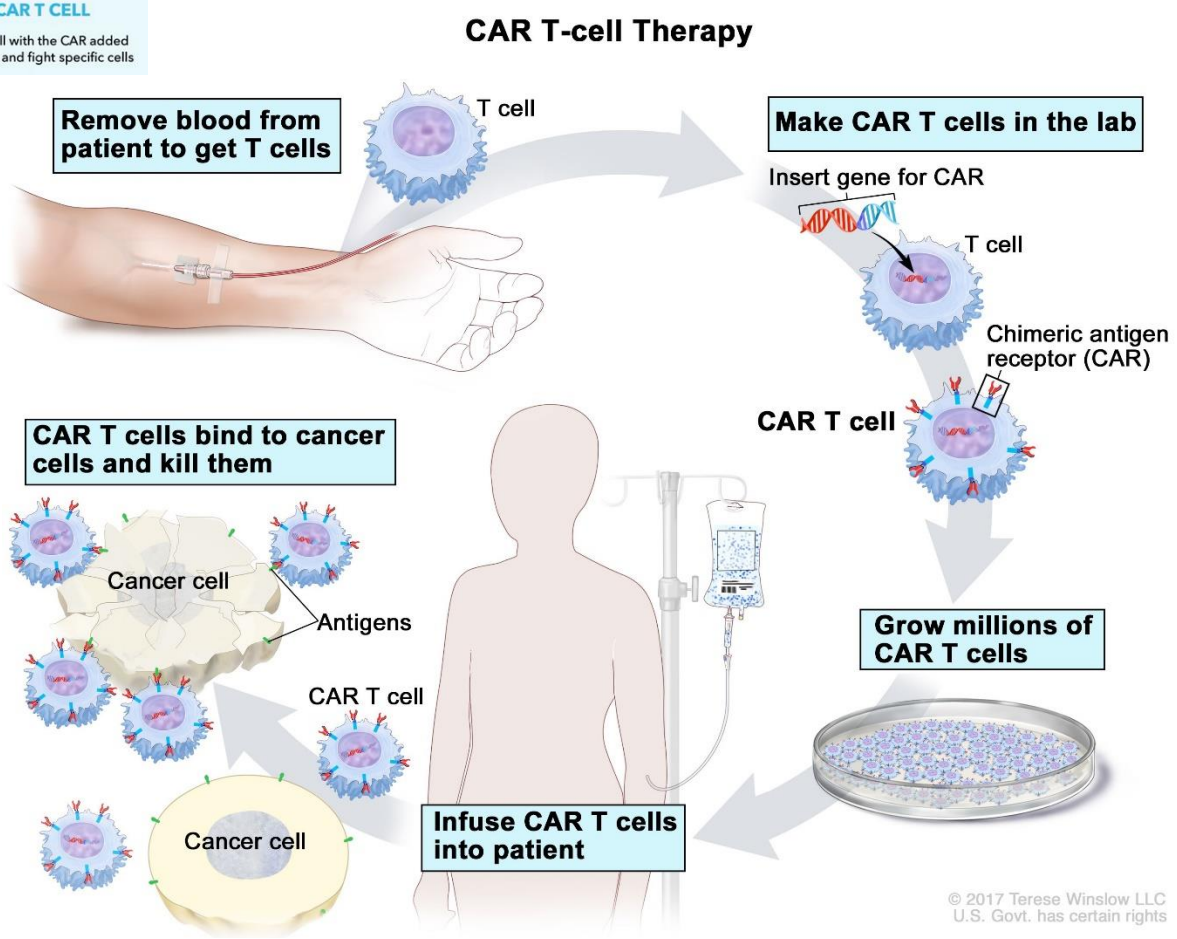
Immunotherapy

Cancer treatment vaccines



Immunotherapy





Thank you for your attention!

Questions?

Please write to carlos.plazasirvent@rub.de