LICENCIATURA EM BIOLOGIA

DISCIPLINA

BIOQUÍMICA

Ano Lectivo de 2013/2014

16ª aula

Proteínas

Imunologia: os anticorpos.

Utilização de anticorpos: investigação científica e prática clínica.

Exemplo: determinação da tipologia sanguínea.

ANTIBODIES
What are they?

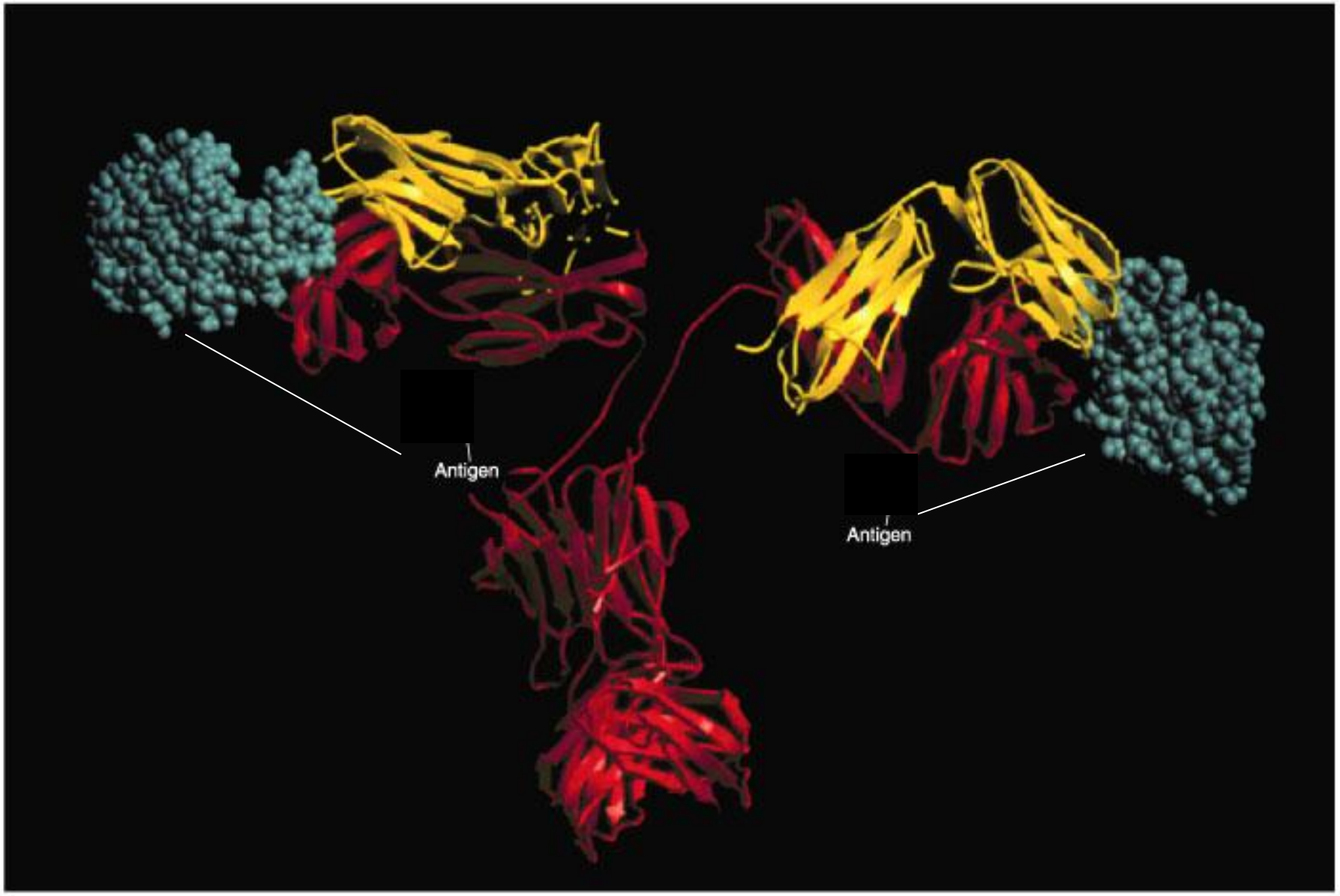
- **Antibodies** are:
  - Proteins
  - Protective agents of the immune system
    - Neutralize foreign agents called **antigens**
  - Essential part of the Adaptive Immune System (AIS)
    - AIS learns to respond to invading pathogens

ANTIBODIES
What are they?

- **Antibodies** are:

  - "Y"-shaped Immunoglobulins (Ig)
    - Comprised of 2 heavy and 2 light chains
  - 5 different types: IgA, IgD, IgE, IgG, IgM
    - Each have a specific role
  - Contain **Variable Regions** which recognize and bind antigen via "lock and key" method

Binding of an antigen by an antibody.



How Antibodies are Generated

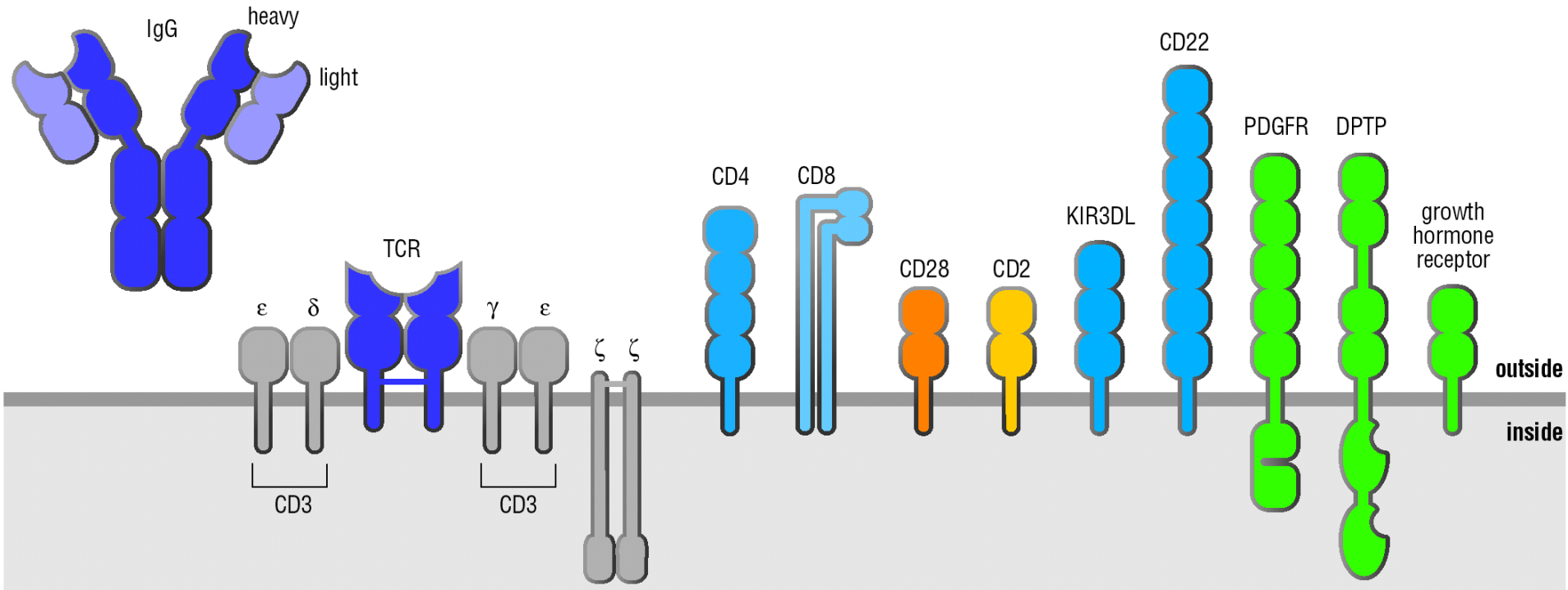
- Antibodies occur in 2 forms

  – Soluble: secreted in blood and tissue

  – Membrane-bound: found on surface of B-cell, also known as a B-cell receptor (BCR)

  - BCR binds circulating antigen, activating the B-cell and forming plasma cells or memory B-cells

The Immunoglobulin Superfamily
a few examples

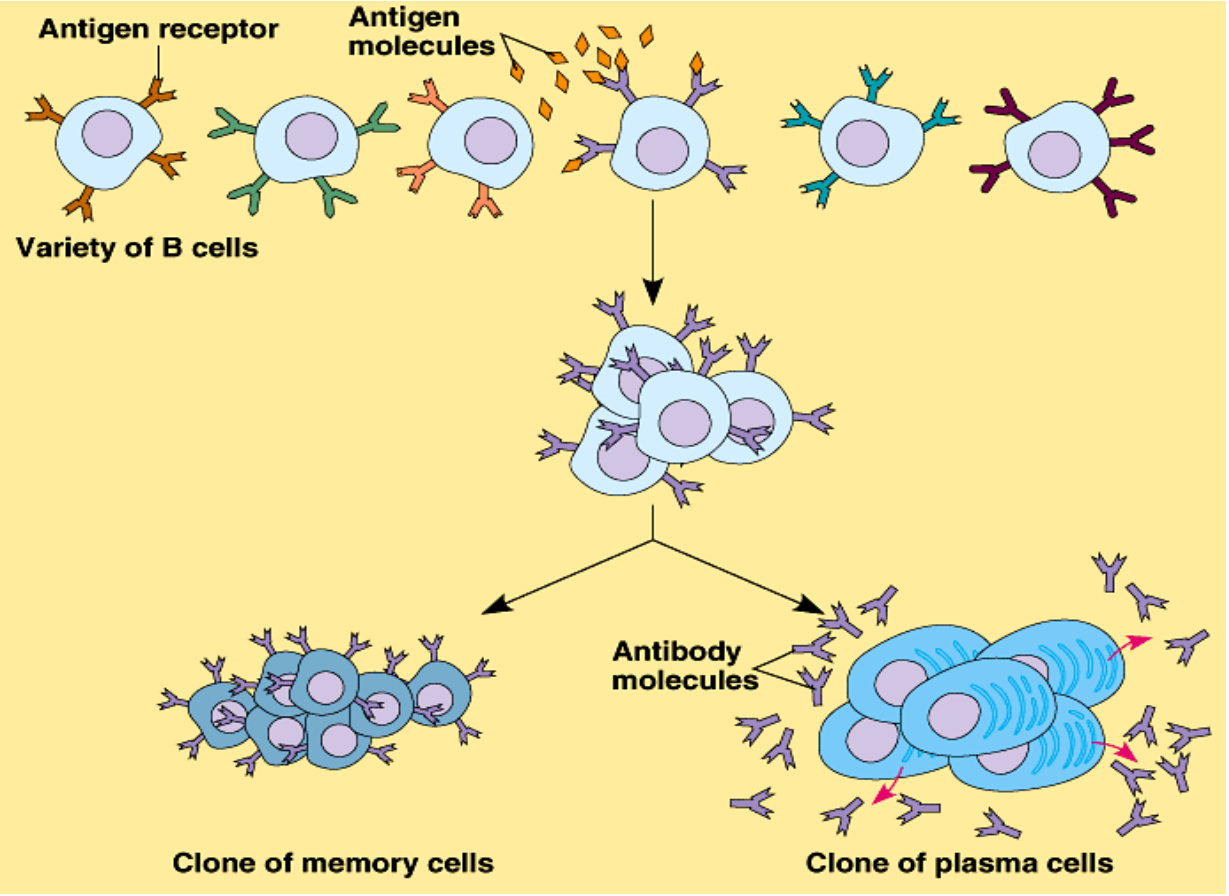


How Antibodies are Generated

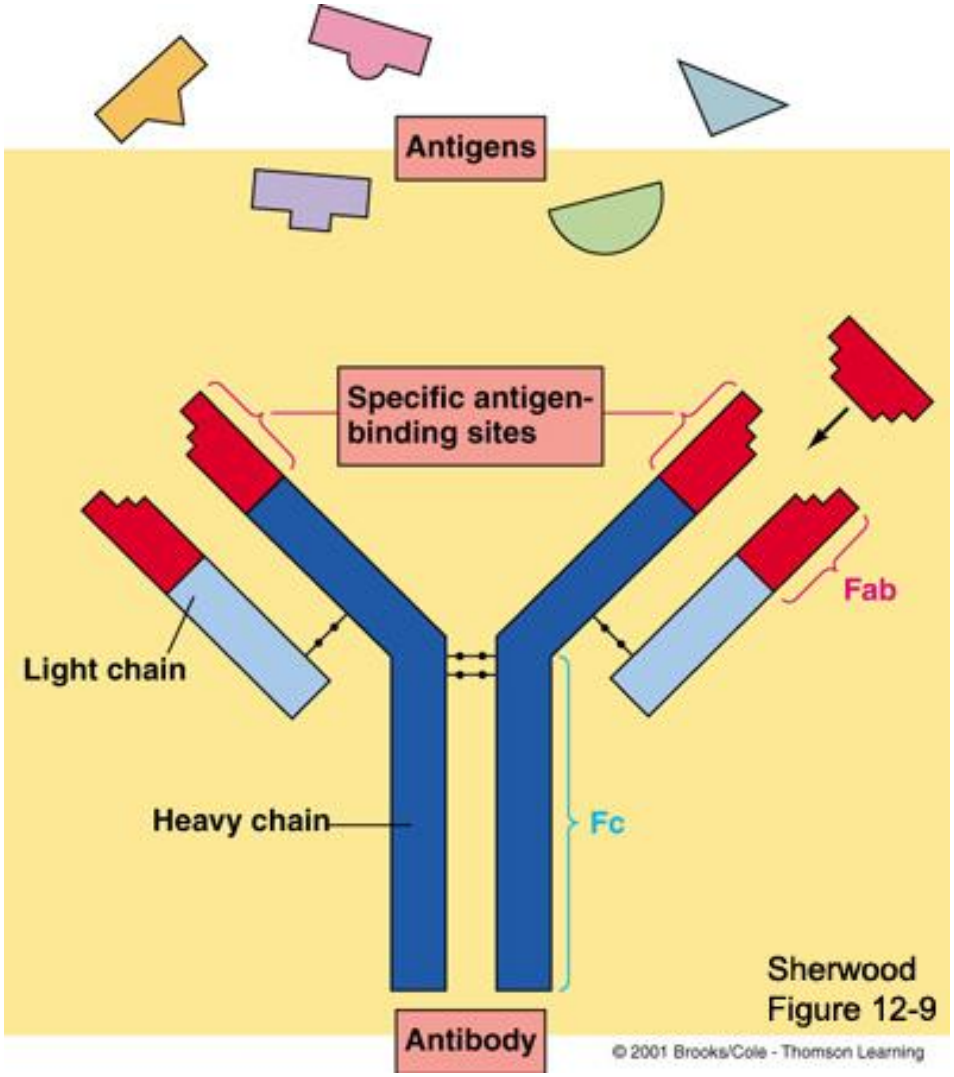
- Activation of a B cell and Clonal Expansion

1) Antigen binds the BCR on a B-cell, activating it

2) B-cell begins to divide (Clonal Expansion), forming either plasma cells (antigen factories) or memory B-cells.

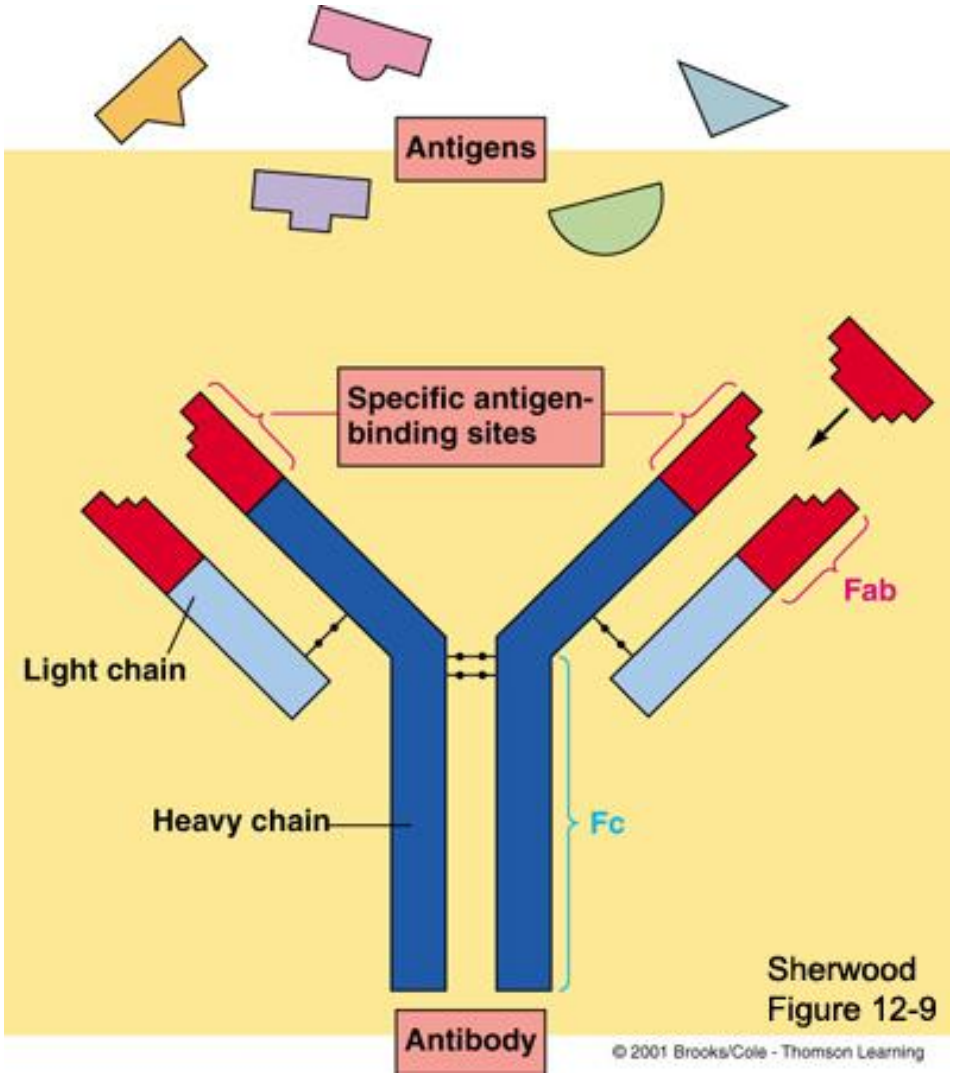


Antibody Structure



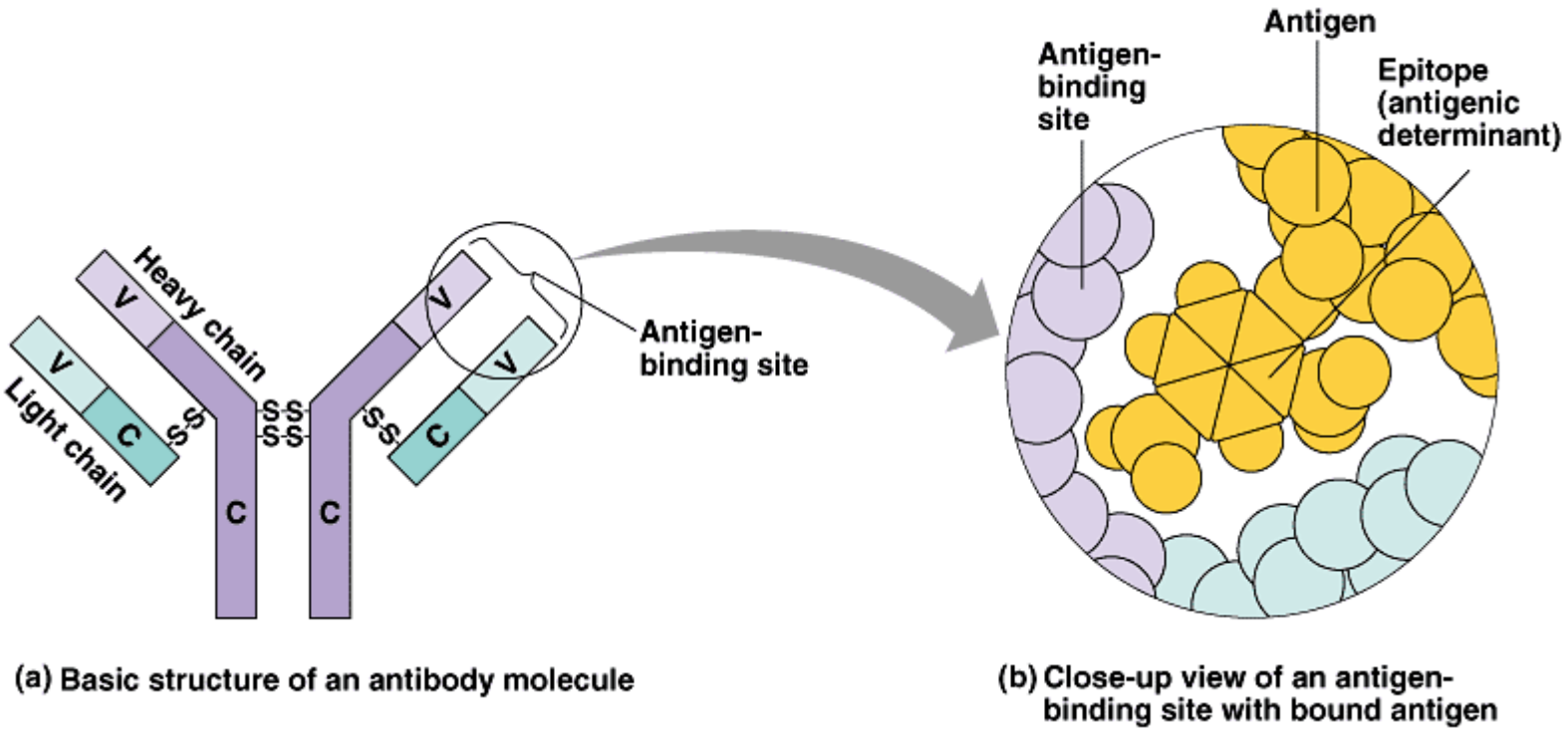
- Antibodies are globular proteins called Immunoglobulins (Ig)
- “Y”-shaped
- Made up of 4 polypeptide chains
  - 2 identical heavy
  - 2 identical light
  - connected by disulfide bonds (-S-S-)

Antibody Structure



- Antibodies can also be divided into two regions based on their function
  - *Fab (fragment, antigen binding) region.*
    - Tip of the antibody
    - Binds the antigen
  - *Fc (fragment, crystallizable) region*
    - Base of the antibody
    - Can bind cell receptors, complement proteins and other molecules

Antibody Structure

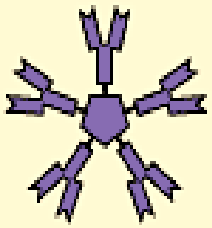


- Each heavy and light chain has a **constant** and **variable** region
- The variable region binds the antigen in a “lock-and-key” manner

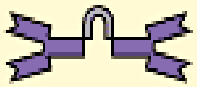
Antibody Isotype

- Mammals express 5 different isotypes of antibodies (IgA, IgD, IgE IgG and IgM) with different functions and locations
- Class of antibody is defined by the heavy chain

Table 43.1 The Five Classes of Immunoglobulins

IgM (pentamer) 	IgMs are the first circulating antibodies to appear in response to an initial exposure to an antigen; their concentration in the blood then declines rapidly. Thus the presence of IgM usually indicates a current infection. IgM consists of five Y-shaped monomers arranged in a pentagonal structure. The numerous antigen-binding sites make it very effective in agglutinating antigens and in reactions involving complement. IgM is too large to cross the placenta and does not confer maternal immunity.
IgG (monomer) 	IgG is the most abundant of the circulating antibodies. It readily crosses the walls of blood vessels and enters tissue fluids. IgG also crosses the placenta and confers passive immunity on the fetus. IgG protects against bacteria, viruses, and toxins in the blood and lymph, and triggers action of the complement system.

Antibody Isotype

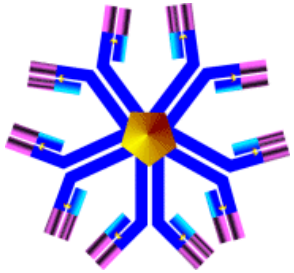
IgA (dimer) 	IgA is produced by cells in mucous membranes. The main function of IgA is to prevent the attachment of viruses and bacteria to epithelial surfaces. IgA is also found in many body secretions, such as saliva, perspiration, and tears. Its presence in the first milk produced helps protect the infant from gastrointestinal infections.
IgD (monomer) 	IgD antibodies do not activate the complement system and cannot cross the placenta. They are mostly found on the surfaces of B cells, probably functioning as antigen receptors that help initiate the differentiation of B cells into plasma cells and memory B cells.
IgE (monomer) 	IgE molecules are slightly larger than IgG and represent only a small fraction of the antibodies in the blood. The tails attach to mast cells and basophils and, when triggered by an antigen, cause the cells to release histamine and other chemicals that cause an allergic reaction.

- Basic antibody is composed of 1 Ig unit, i.e. is a **monomer**
- Some are dimeric (IgA) or pentameric (IgM)
- Isotype changes during development of B-cell

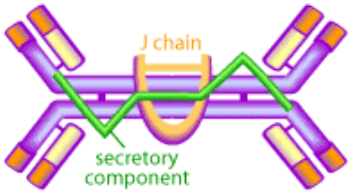
Antibody Isotype

- Immature B-cells only express surface IgM
- As it matures, it expressed both IgM and IgD
- After reaching maturity, the B-cell is ready to interact with antigen and produce antibody
- As antibodies are formed, some undergo isotype switching and produce IgE, IgA or IgG

Antibody Isotype



- IgM – 1st class of circulating antibody
  - found in pentameric form
- IgG - most abundant antibody
- IgA - located in the mucous membranes
  - found in dimeric form



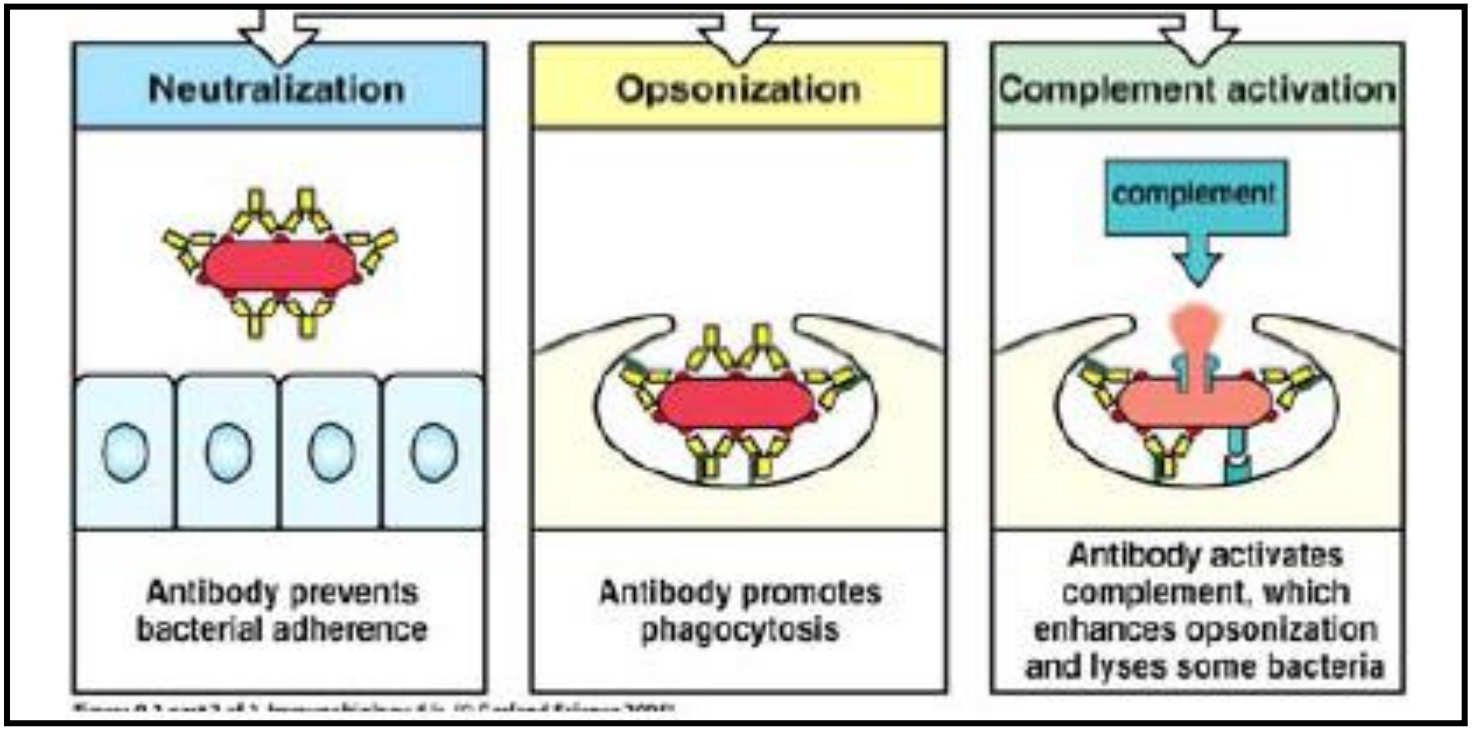
- IgD - found on surface of B-cells
  - probably involved in memory cell formation
- IgE - involved in allergies, i.e. trigger release of histamine

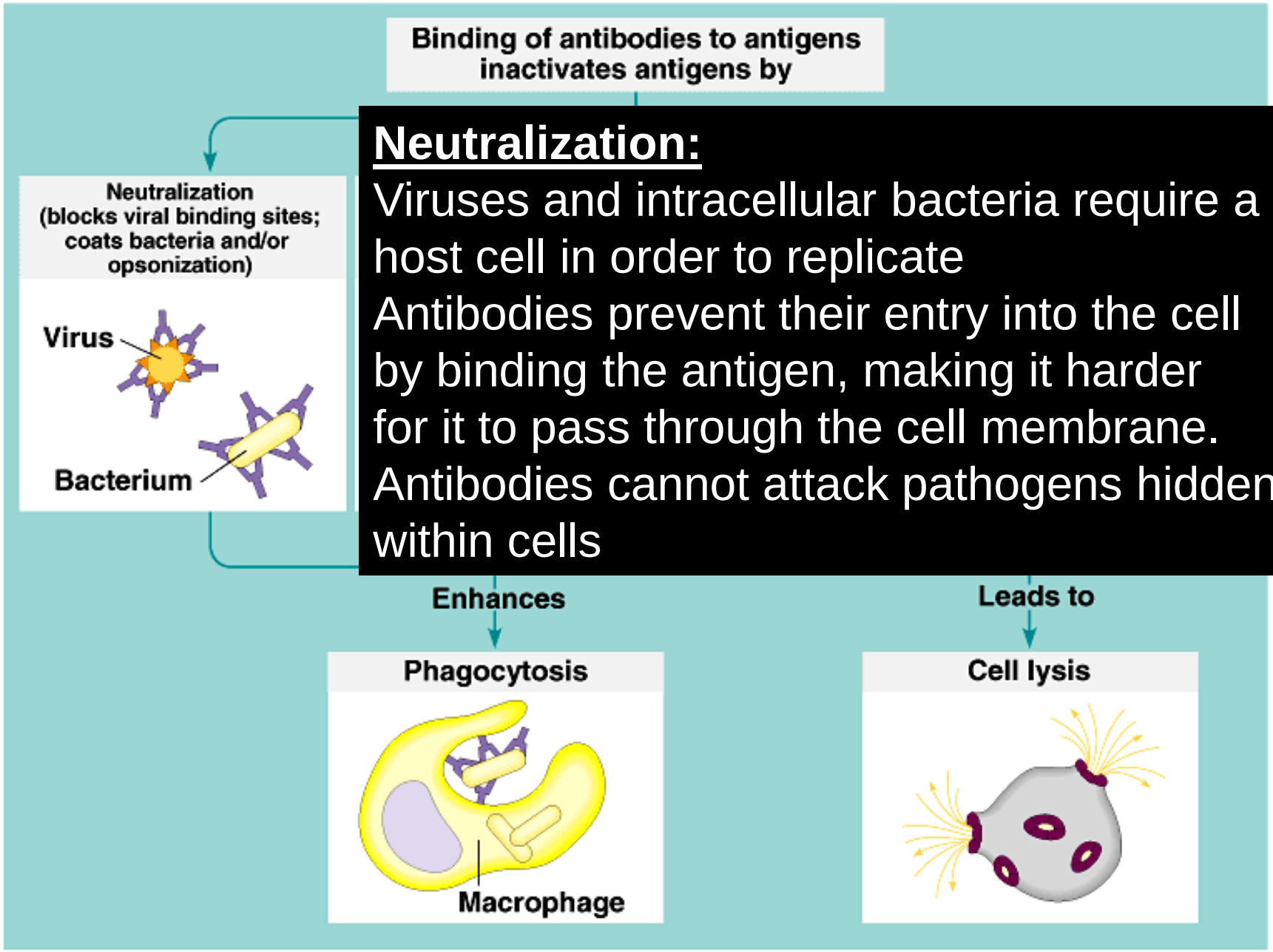
Major functional properties of antibodies

Antibody class	Major Functional properties
IgM	complement activation; antigen trapping; antigen receptor of naïve B cells
IgG	complement activation, phagocytosis, ADCC, transfer of adaptive immunity to offspring, regulation of antibody production
IgA	mucosal immunity, phagocytosis
IgE	activation of mast cells, basophils, eosinophils
IgD	antigen receptor on naïve B cells

Antibody Function

- Antibodies are the main component of the Humoral Immune System
- They bind antigen and flag them for elimination via 1 of 3 ways:



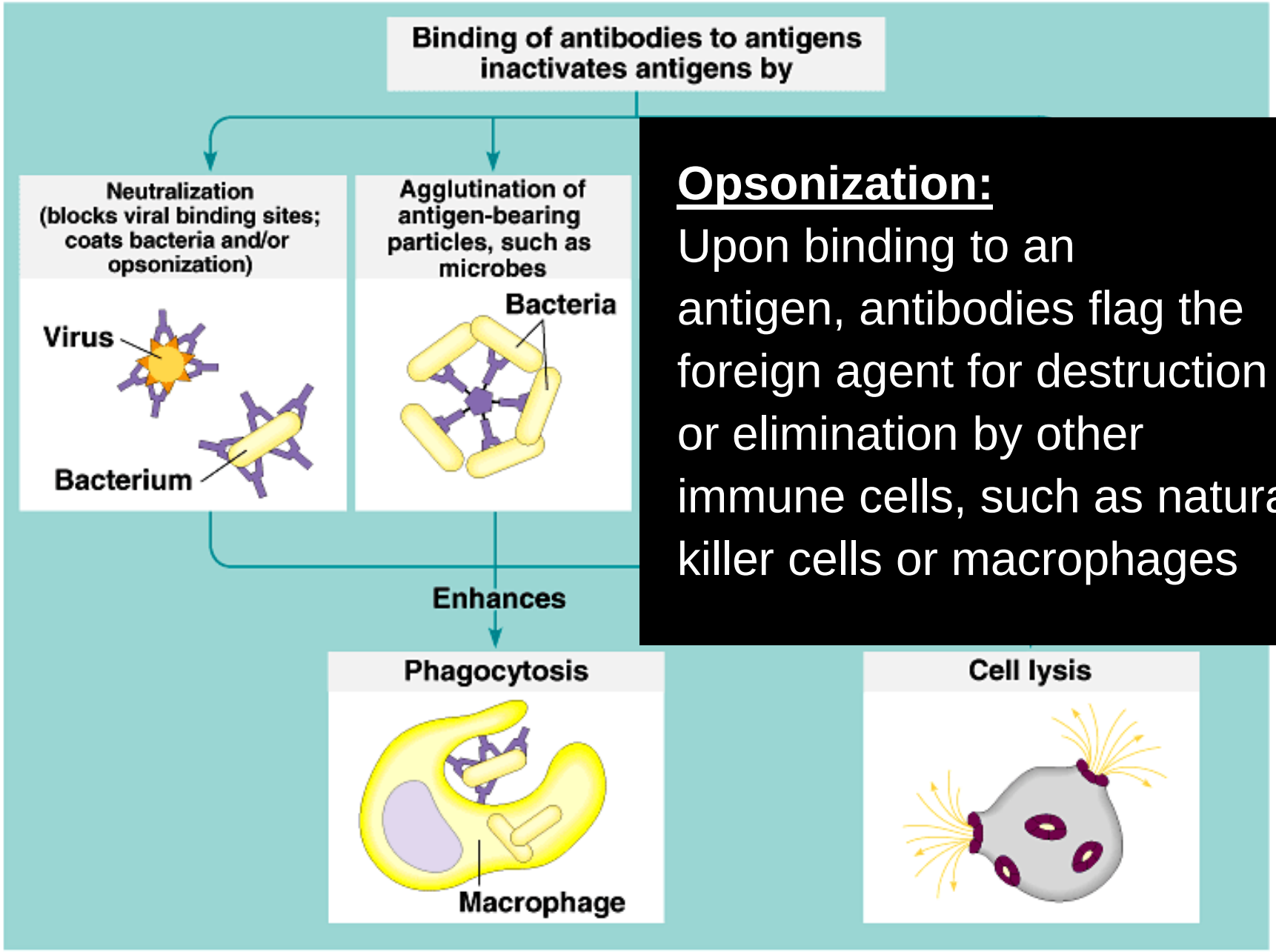


Neutralization:

Viruses and intracellular bacteria require a host cell in order to replicate

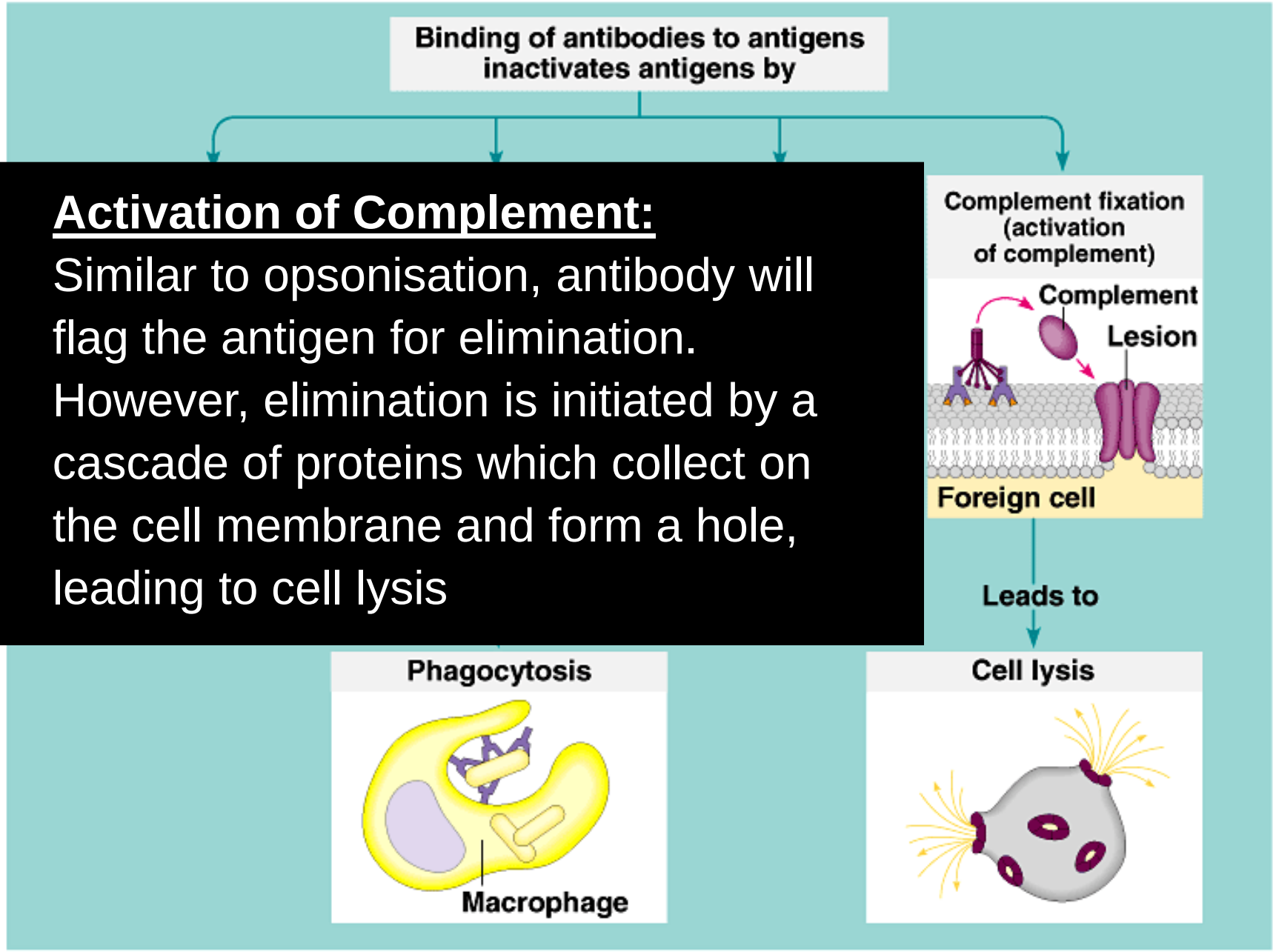
Antibodies prevent their entry into the cell by binding the antigen, making it harder for it to pass through the cell membrane.

Antibodies cannot attack pathogens hidden within cells



Opsonization:

Upon binding to an antigen, antibodies flag the foreign agent for destruction or elimination by other immune cells, such as natural killer cells or macrophages



Activation of Complement:

Similar to opsonisation, antibody will flag the antigen for elimination. However, elimination is initiated by a cascade of proteins which collect on the cell membrane and form a hole, leading to cell lysis

Affinity and Avidity

•**Affinity**: the strength of binding between a single binding site and a single ligand.

$$K_D = \frac{[A][B]}{[AB]}$$

•**Avidity**: the strength of binding between a molecule and a complex ligand, e.g. if there are multiple binding sites then the avidity may be increased by increasing the number of binding sites or by increasing the affinity of those binding sites.

Immunoglobulin genes

Generation of Ig diversity in B cells
before encounter with antigen (Primary Repertoire)

- the body can produce billions of different antibodies (although a single B cell produces one specificity only)
- part of this diversity is produced by the various combinations of H and L chain polypeptides
- no ready made genes in the germ line
- Ig heavy and light chain loci consist of families of gene segments. Some of these segments are rearranged to somatically generate the immunoglobulin genes in B lymphocytes (only)
- the rearrangement process generates the enormous diversity

ANTIBODIES
summary

- Secreted by B lymphocytes
- Great diversity and specificity: $>10^9$ different antibodies; can distinguish between very similar molecules
- Tag particles for clearance/destruction
- Protect against re-infection (vaccines)

Antibody-based assays

Cell cycle analysis of cultured newt lung cells

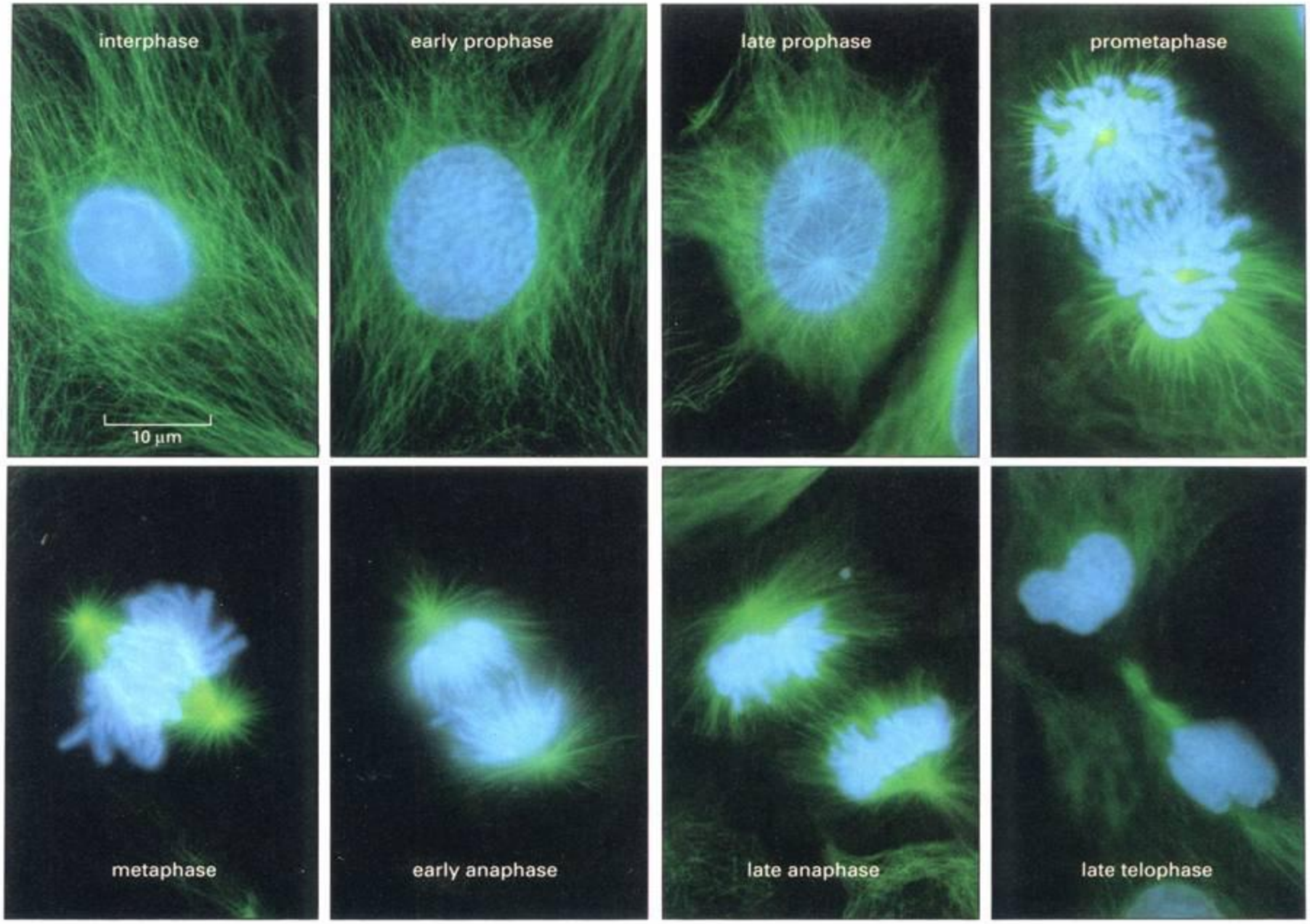


TABLE 6-3 Sensitivity of various immunoassays

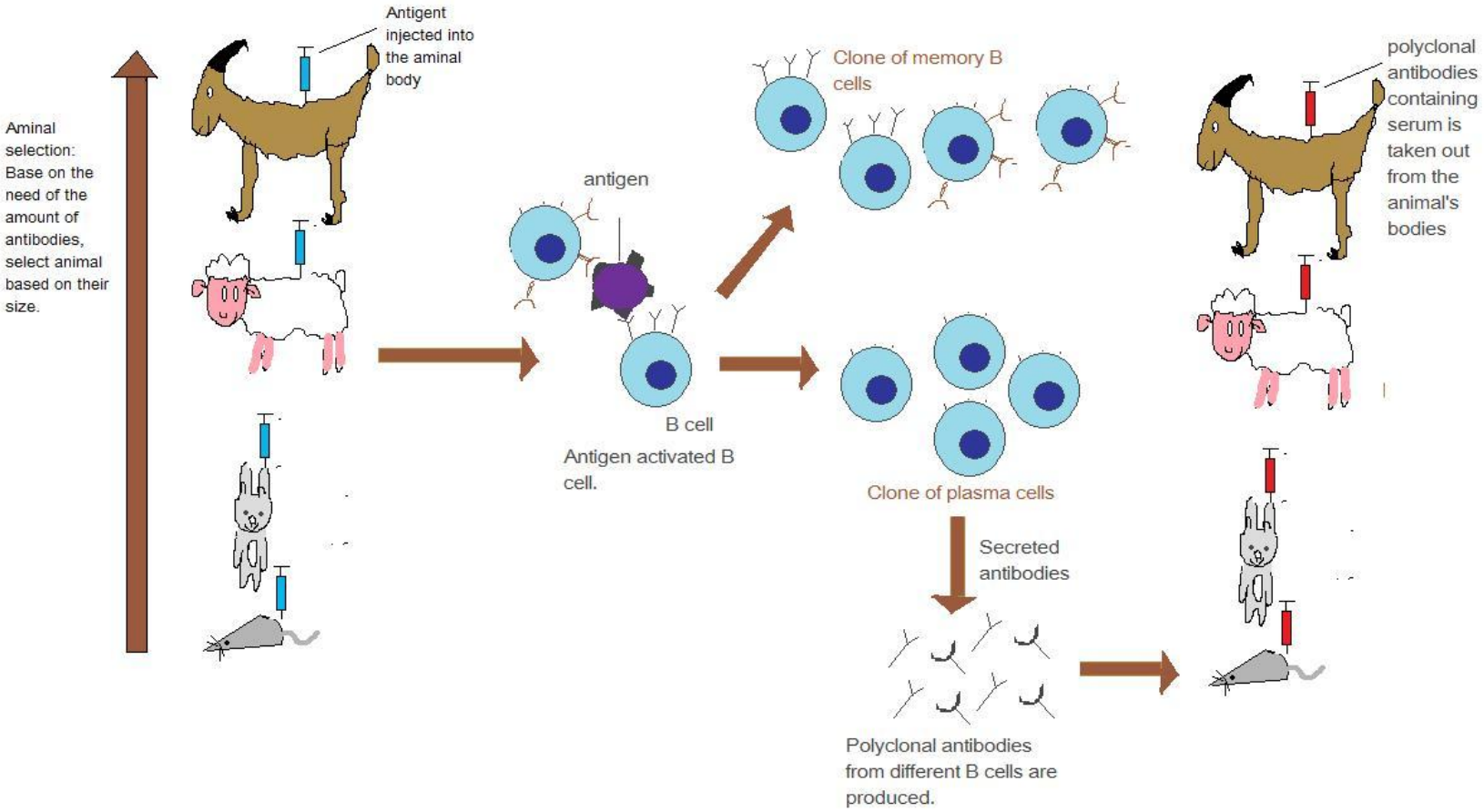
Assay	Sensitivity* (μg antibody / ml)
Precipitation reaction in fluids	20–200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10–50
Ouchterlony double immunodiffusion	20–200
Immunoelectrophoresis	20–200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	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

Polyclonal Antibodies



Monoclonal antibodies

- Single antibody (all same H and L chains)
- Made by fusion of B cells to a transformed cell line of the plasma cell type and selection for “hybridomas” that produce antibody with the desired properties
- Standardized, unlimited reagent for diagnosis or therapy (human antibodies or “humanized” antibodies can be made)

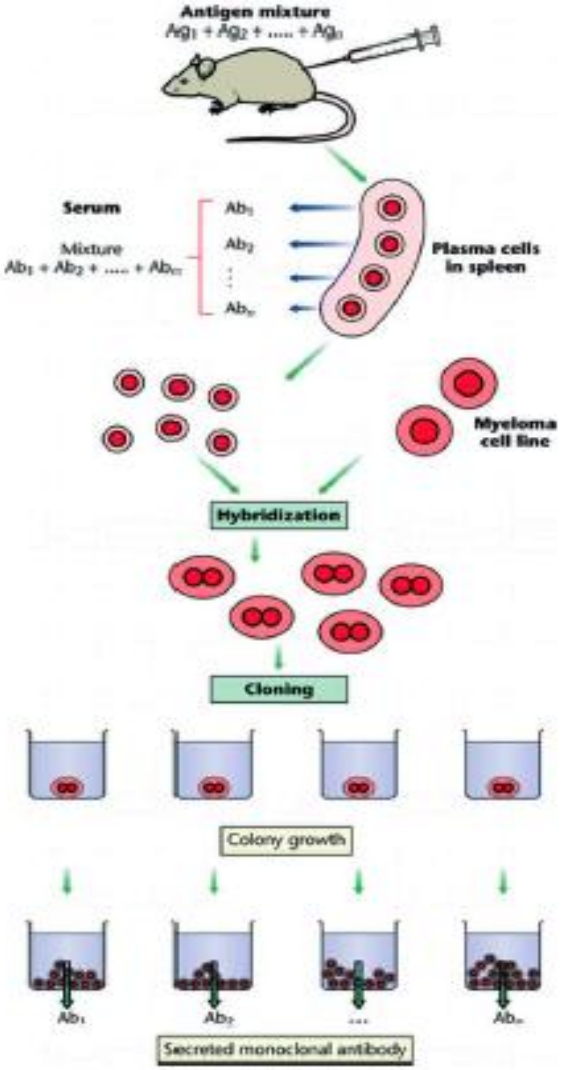
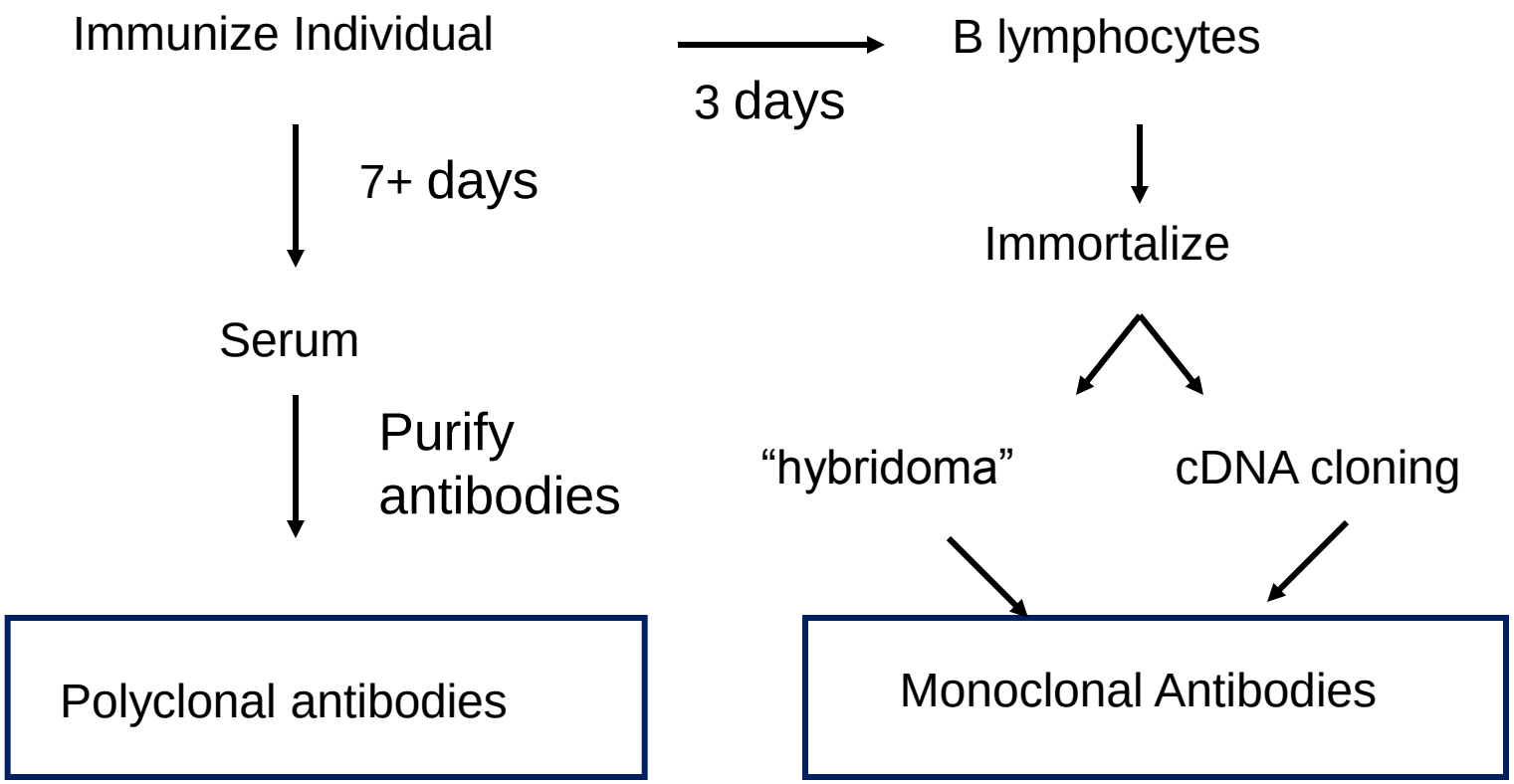


Figure 1 Schematic representation of the process of immortalizing an antibody-producing clone by hybridization, cloning and selection of clones producing the desired antibodies.

Polyclonal vs. Monoclonal Antibodies



Polyclonal vs. Monoclonal antibodies

Monoclonal Ab (mAb) ----

antibody produced from a single clone of B cells
in immunized animal (mouse)
(against only one specific epitope)

Polyclonal Ab ----

antibodies produced from multiple clones of B cells
in immunized animal (mouse, rabbit, goat, horse)
(against many epitopes)

Monoclonal antibodies used in medicine

Standardized, unlimited amounts of reagents for diagnosis or therapy
(human antibodies or “humanized” antibodies can be made).

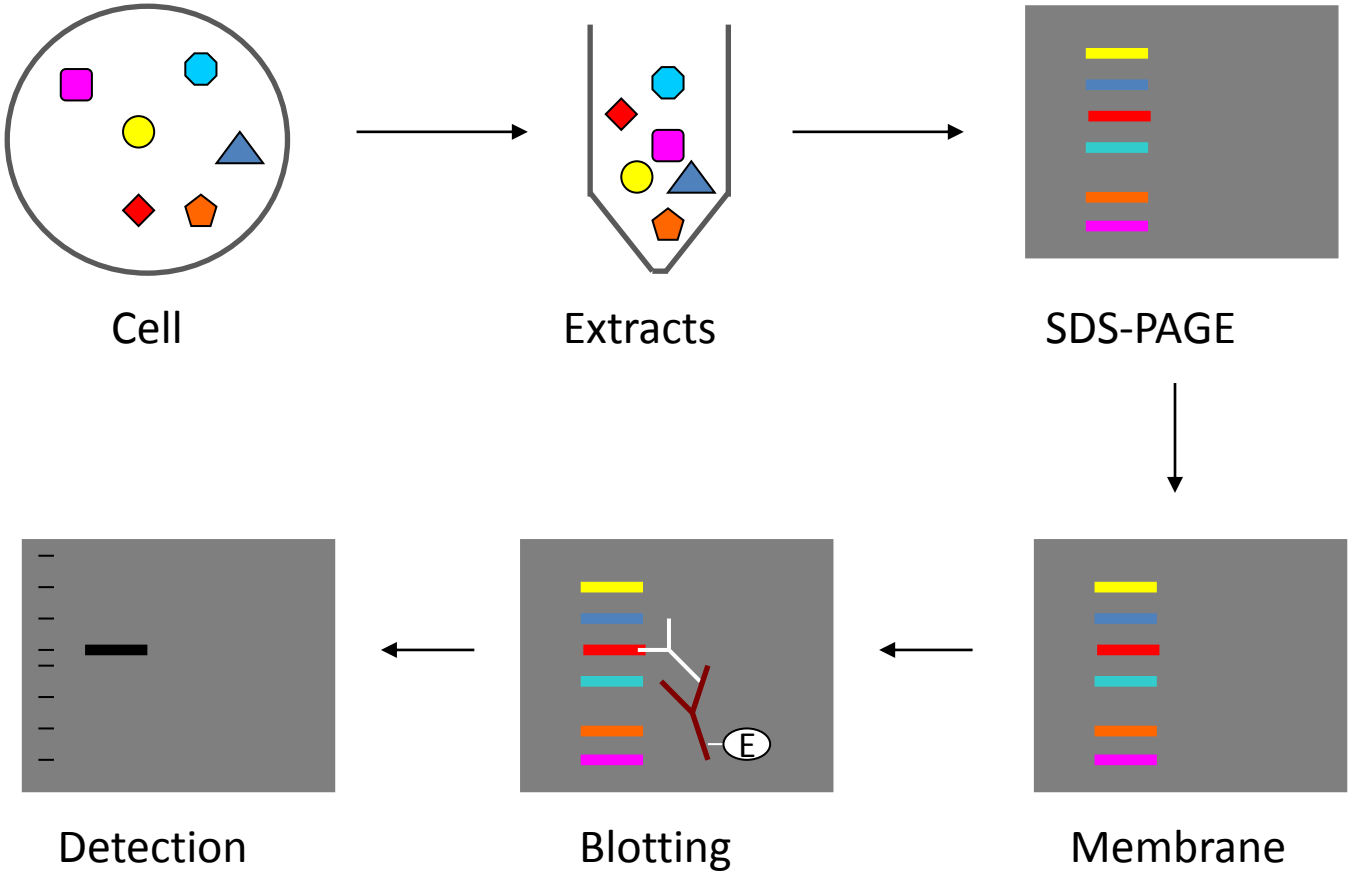
Monoclonal Antibodies Used in Therapies		
monoclonal antibody	**target**	**disease**
trastuzumab	HER2	breast cancer
infliximab	TNF	rheumatoid arthritis, Crohn's disease
rituximab	CD20	non-Hodgkin's lymphoma
abciximab	GPIIb/IIIa	coronary disease
OKT3	CD3	graft rejection

Polyclonal vs. Monoclonal antibodies

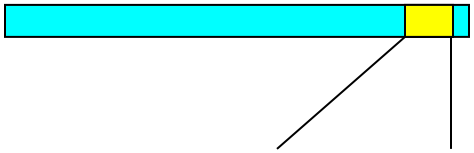
Immunochemical Techniques, Polyclonal versus Monoclonal Antibodies

Technique	Polyclonal antibodies	Monoclonal antibodies	Pooled monoclonal antibodies
Cell Staining	Usually good	Antibody dependent	Excellent
Immunoprecipitation	Usually good	Antibody dependent	Excellent
Immunoblots	Usually good	Antibody dependent	Excellent
Immunoaffinity Purification	Poor	Antibody dependent	Poor
Immunoassays			
Labeled Antibody	Difficult	Good	Excellent
Labeled Antigen	Usually good	Antibody dependent	Excellent

1. Western Blot --- detection of specific antigen in tissue/cell extracts

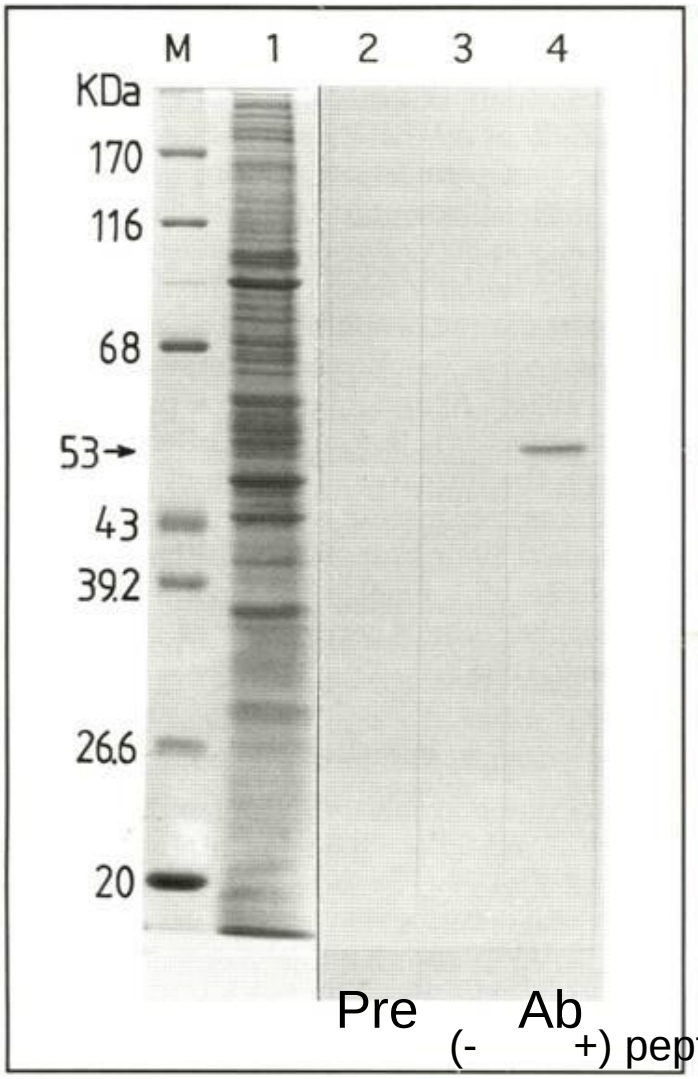


Anti-peptide Ab
of human GSK3a
(53 kDa)



462-475

WB in A431 cell extracts



2. ELISA --- detection of specific antibodies in tissue/cell extracts

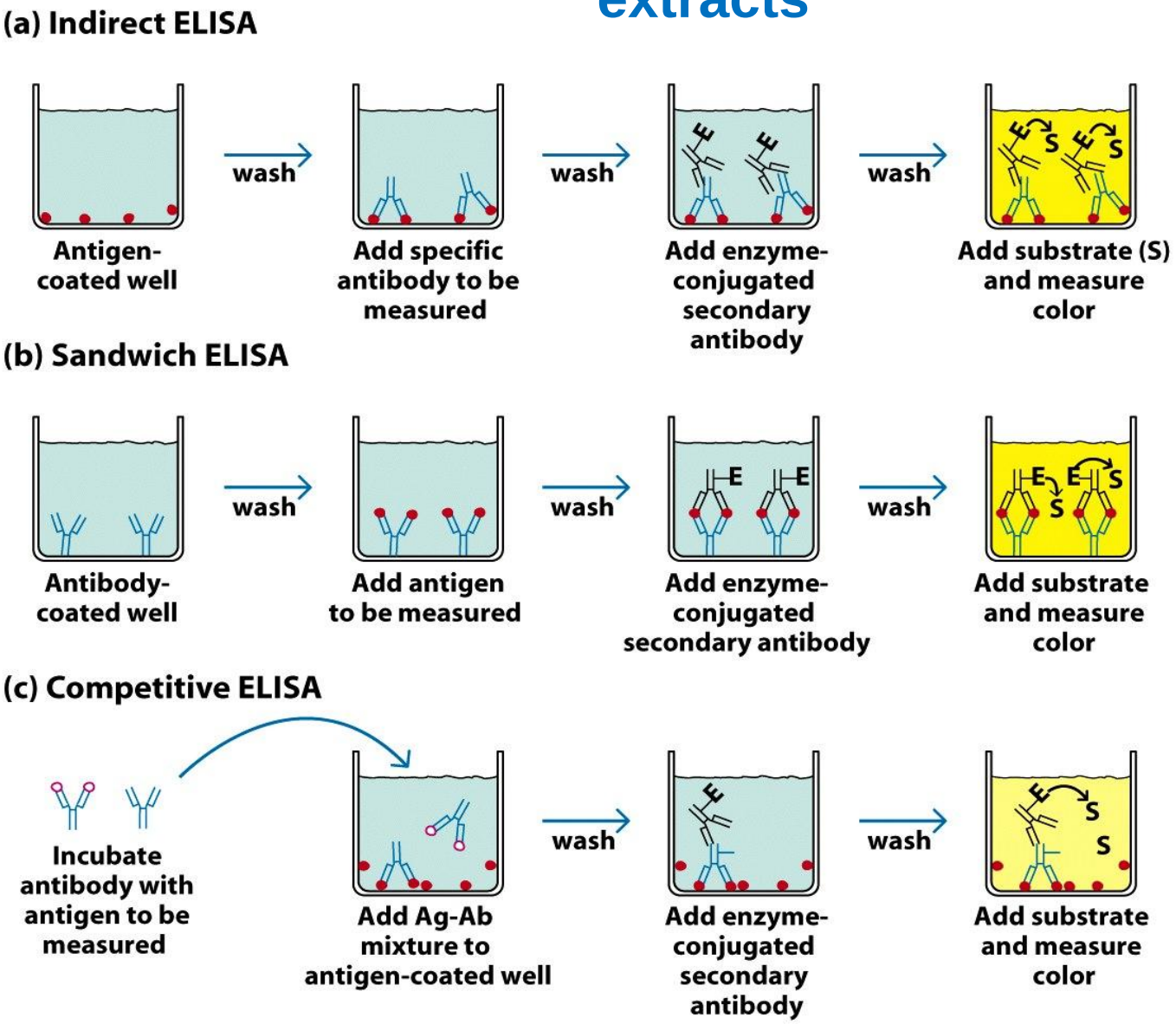


Figure 6-10
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3- Flow cytometry

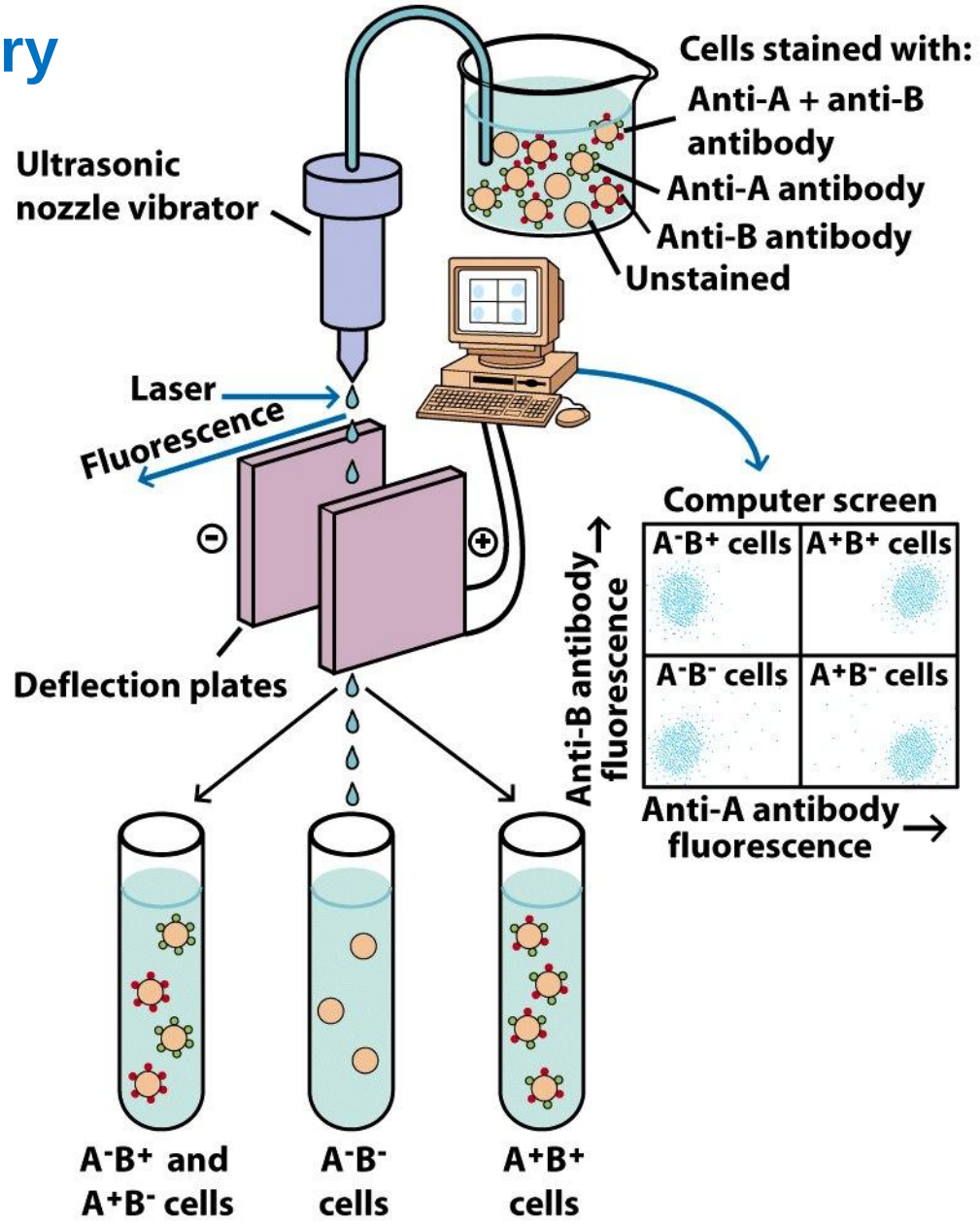

Figure 6-15
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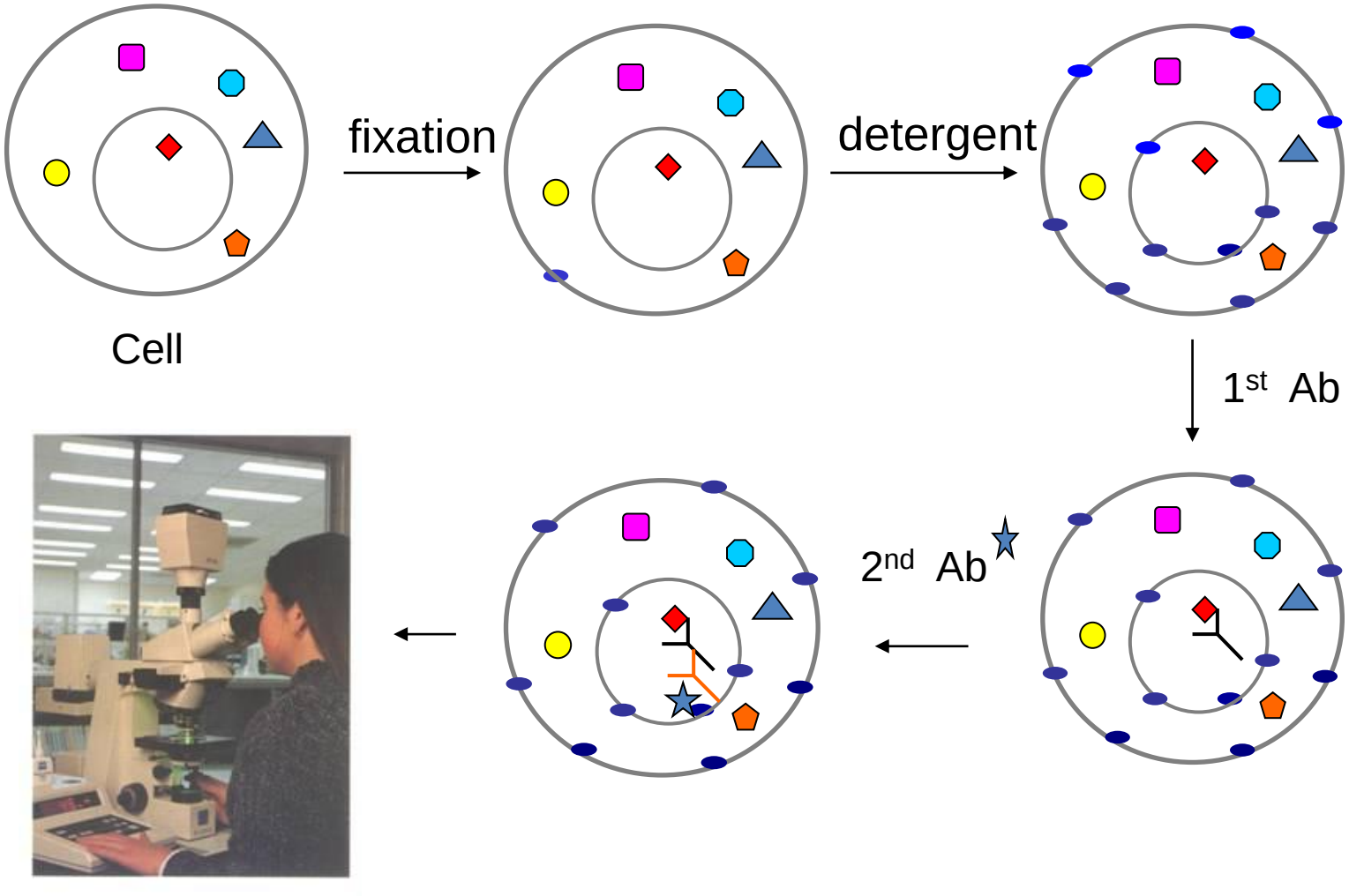
4. Immunofluorescence--- localization of specific antigen in tissue/cells



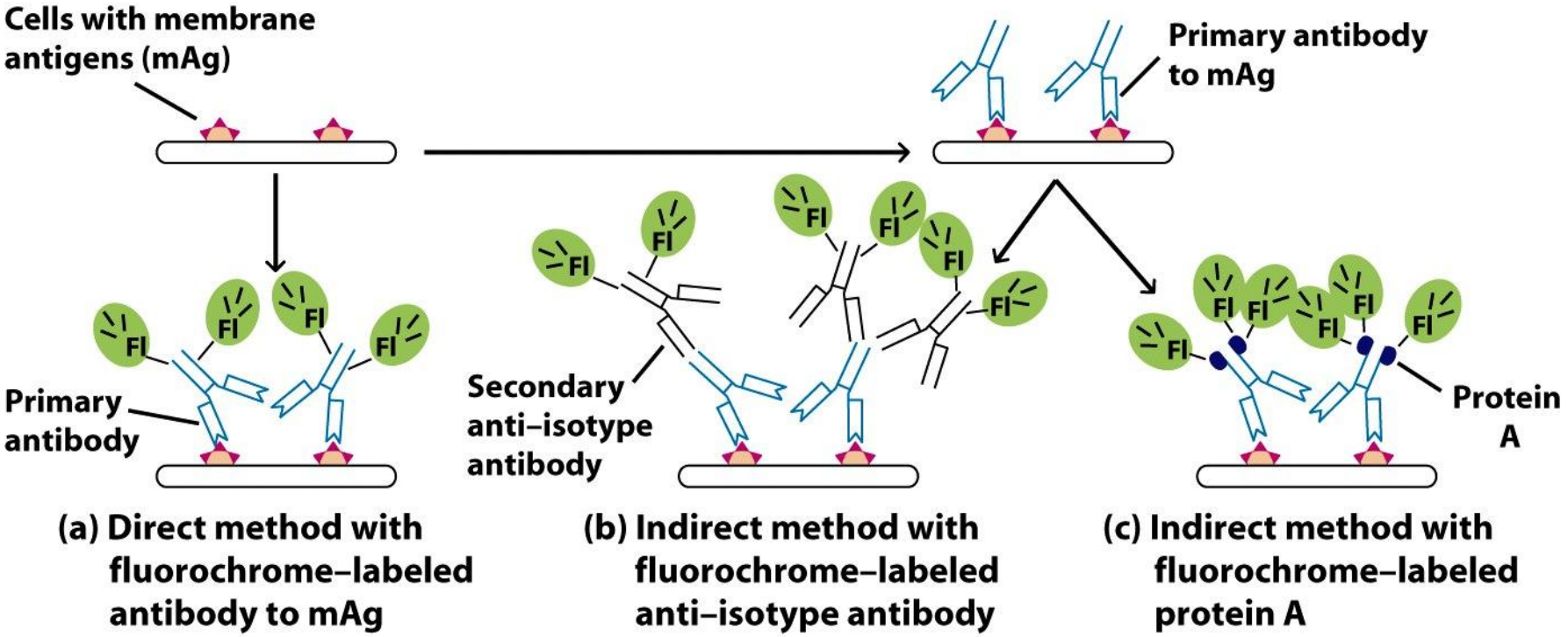
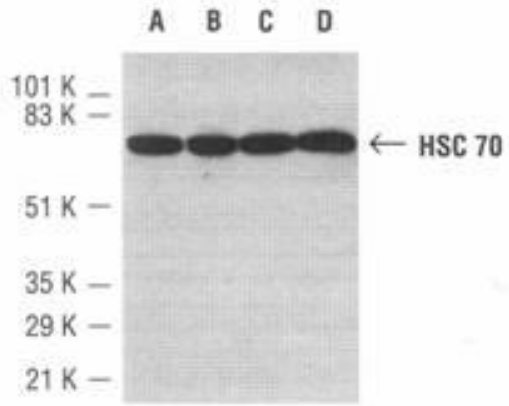


Figure 6-14abc
Kuby IMMUNOLOGY, Sixth Edition

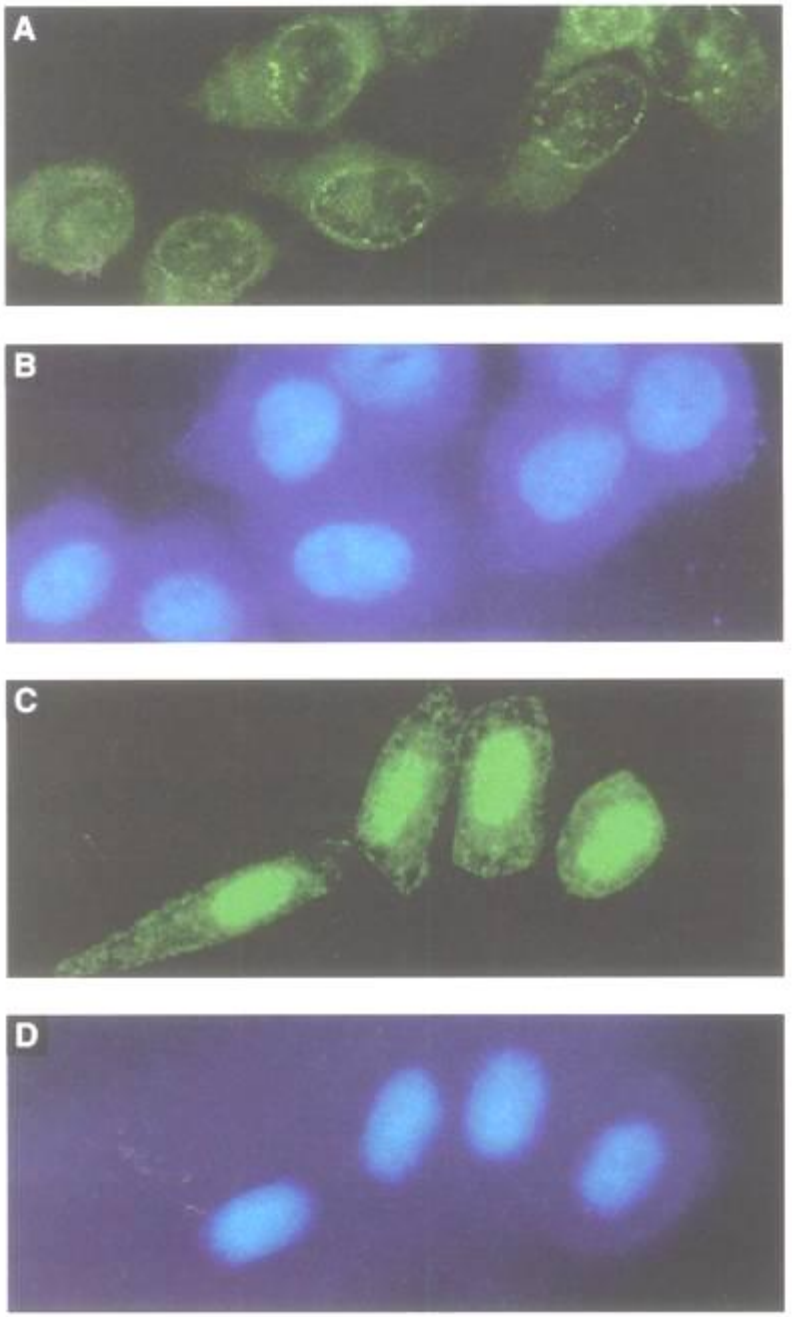
Mouse mAb
of human HSP 70
(70 kDa)



HSC 70 (K-19): sc-1059. Western blot analysis of HSC 70 expression in untreated (A,C) and heat shock activated (B,D) NIH/3T3 (A,B) and HeLa (C,D) whole cell lysates.

Control

Heat
shock



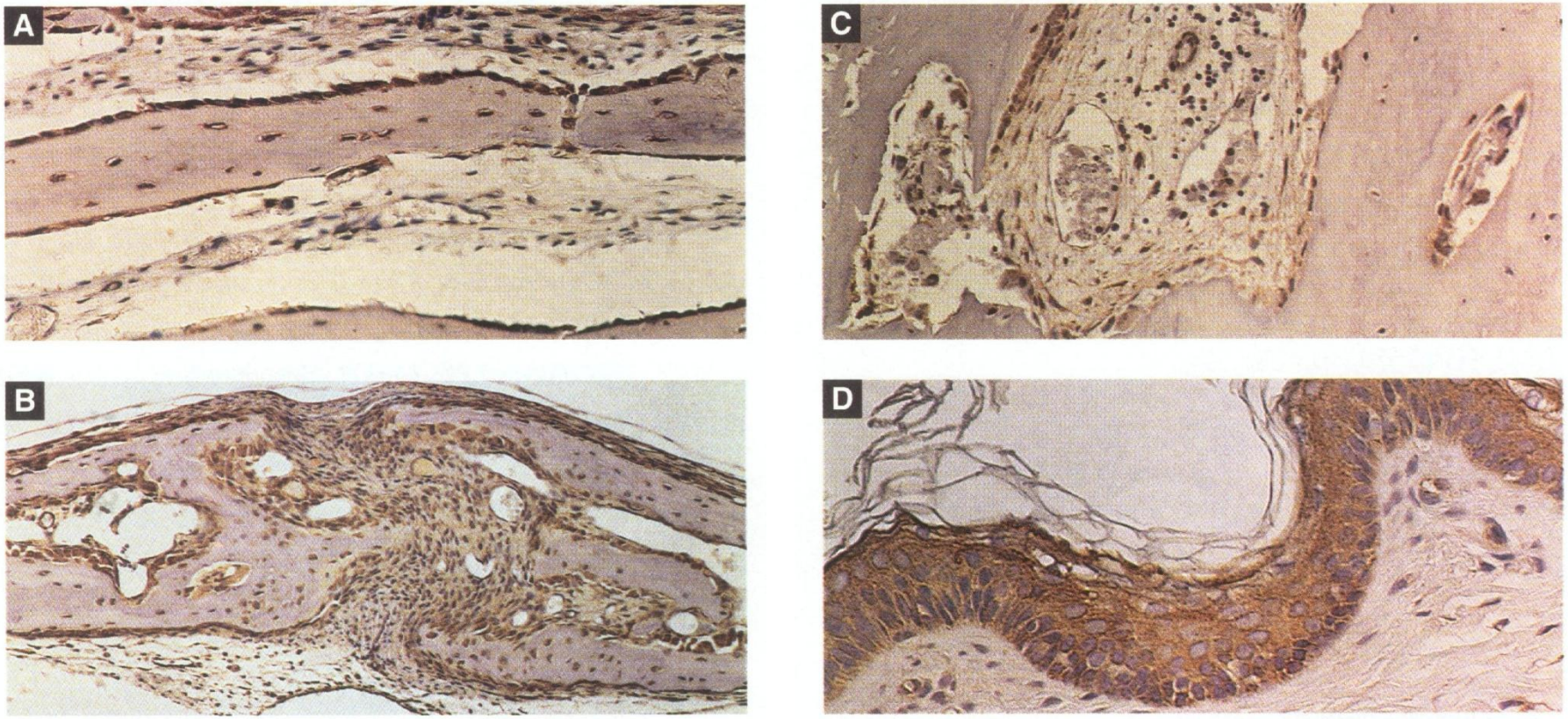
HeLa cells

Anti-peptide Ab
of active caspase-3

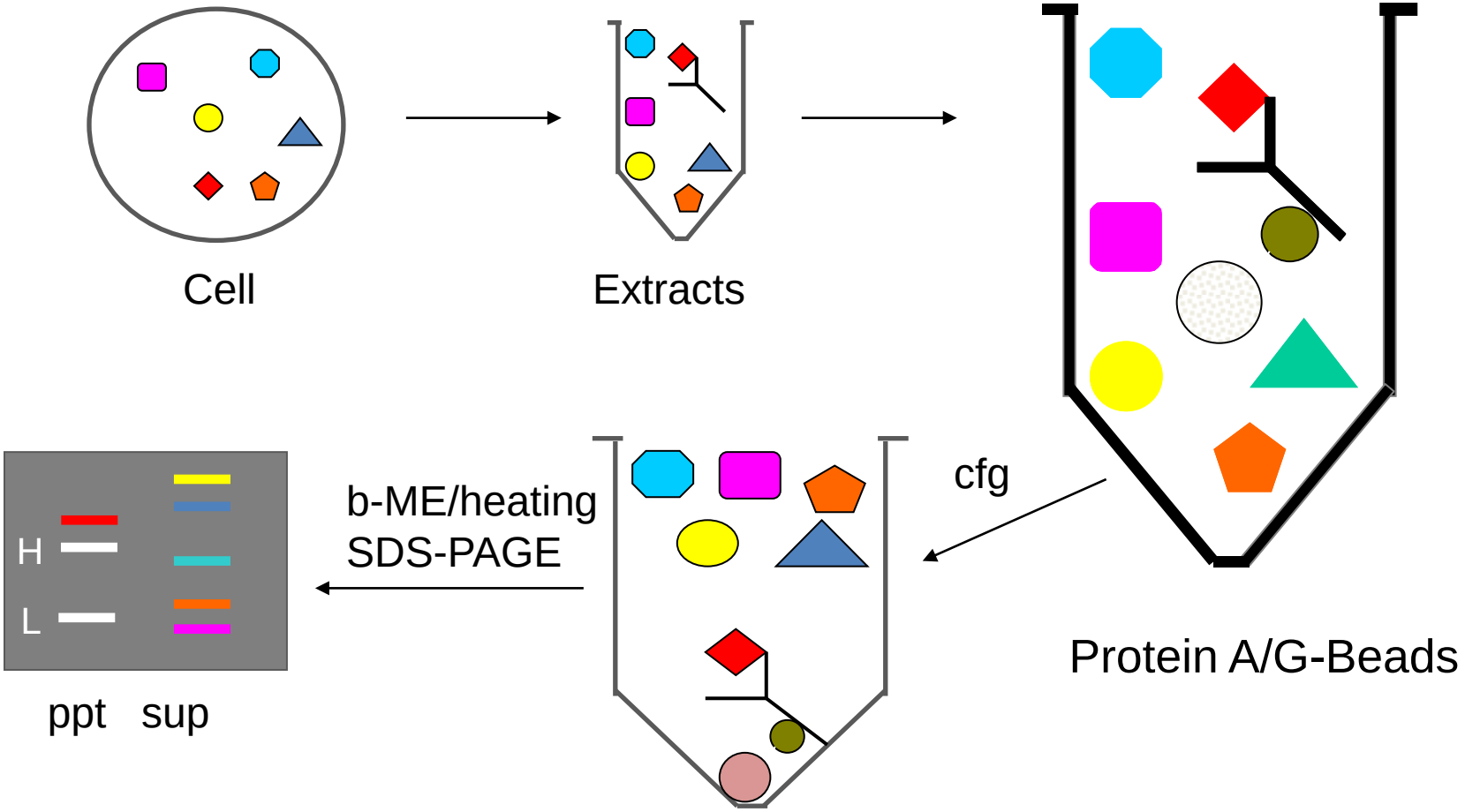
Newborn rat brain tissue/transient ischemia
Double staining with PI (red) and anti-active caspase-3 (green)

5. Immunohistochemistry--- localization of specific antigen in tissues

Anti-peptide Abs
of FGF receptor 1-4



6. Immunoprecipitation--- quickly isolation of specific antigen from tissue/cell extracts (1)



6- Immunoprecipitation (2)

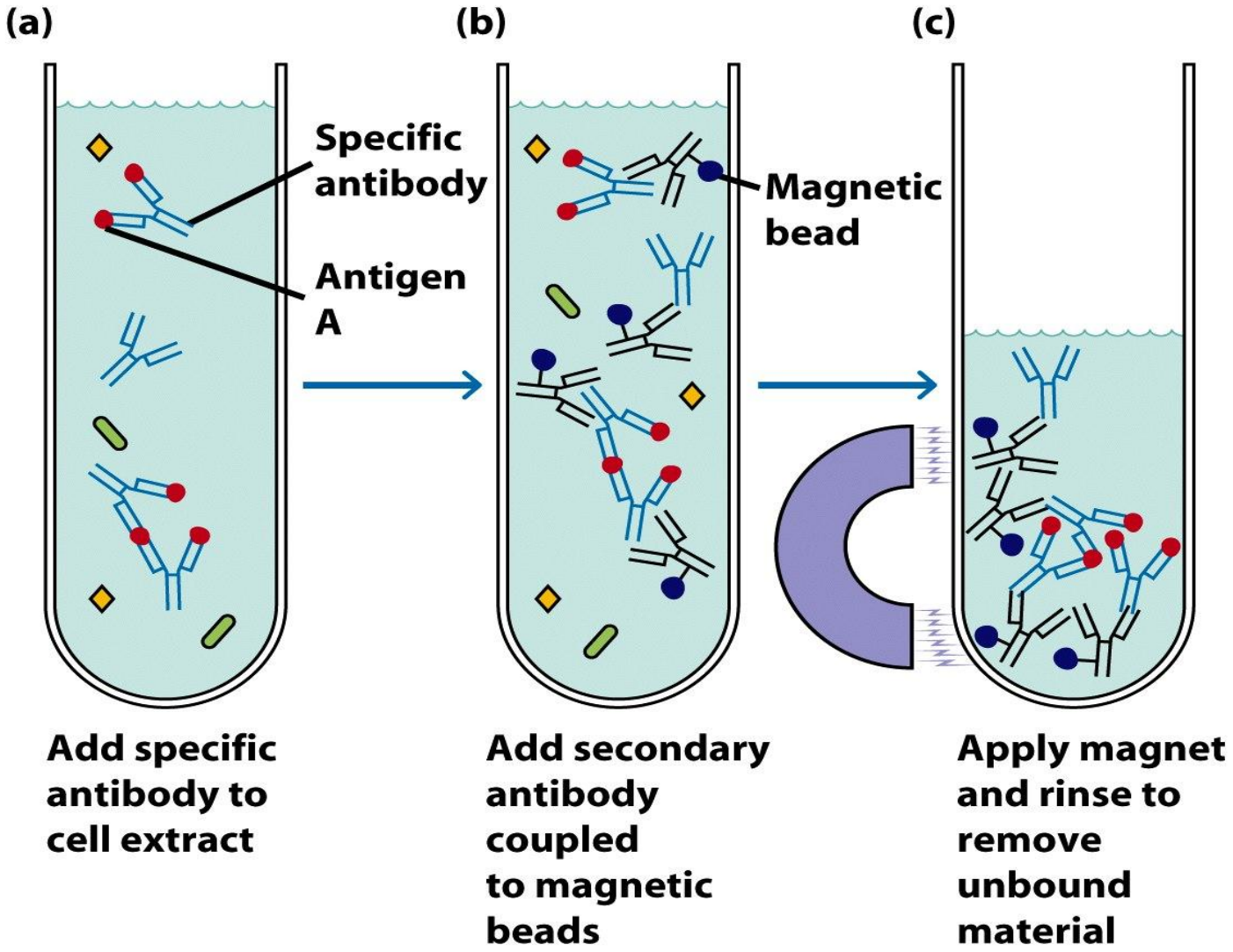


Figure 6-13abc
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To identify proteins that interact with a specific gene product X in cells

A431 cell extracts (10 mg proteins)

5 mg of mAb against X (32 kDa)

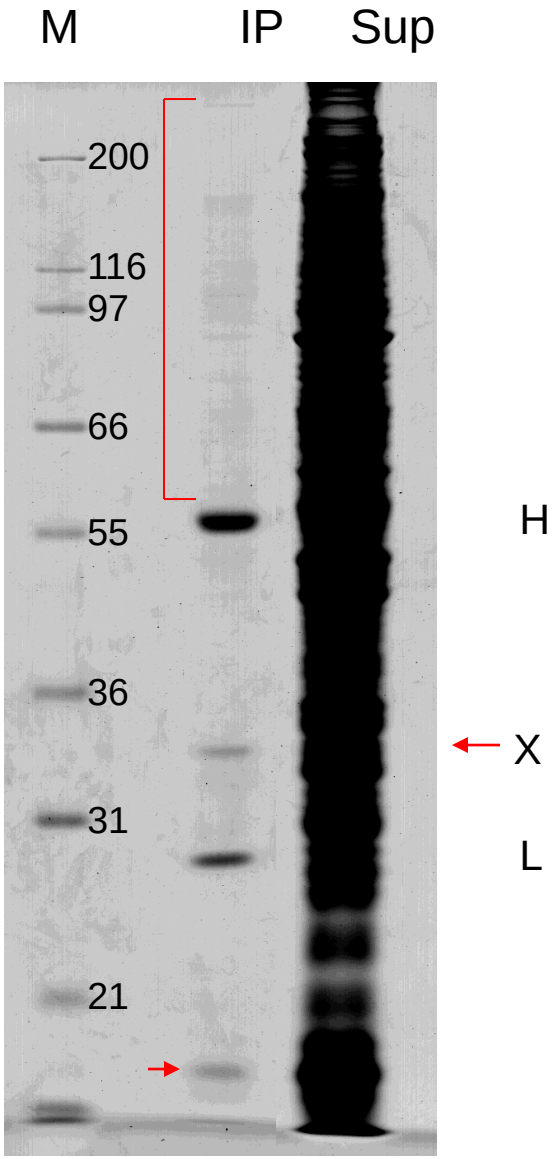
incubation at 4°C for 1.5 h

incubation with protein A-beads at 4°C for 1.5 h

cfg, wash, collect IP

12.5 % SDS-PAGE, Comassie blue stain

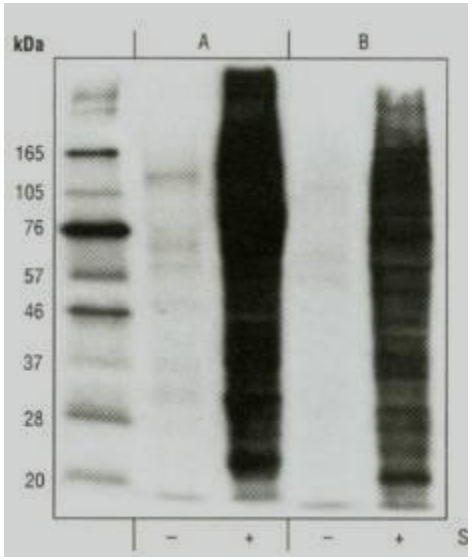
MALDI-TOF MS analysis of bands of interest

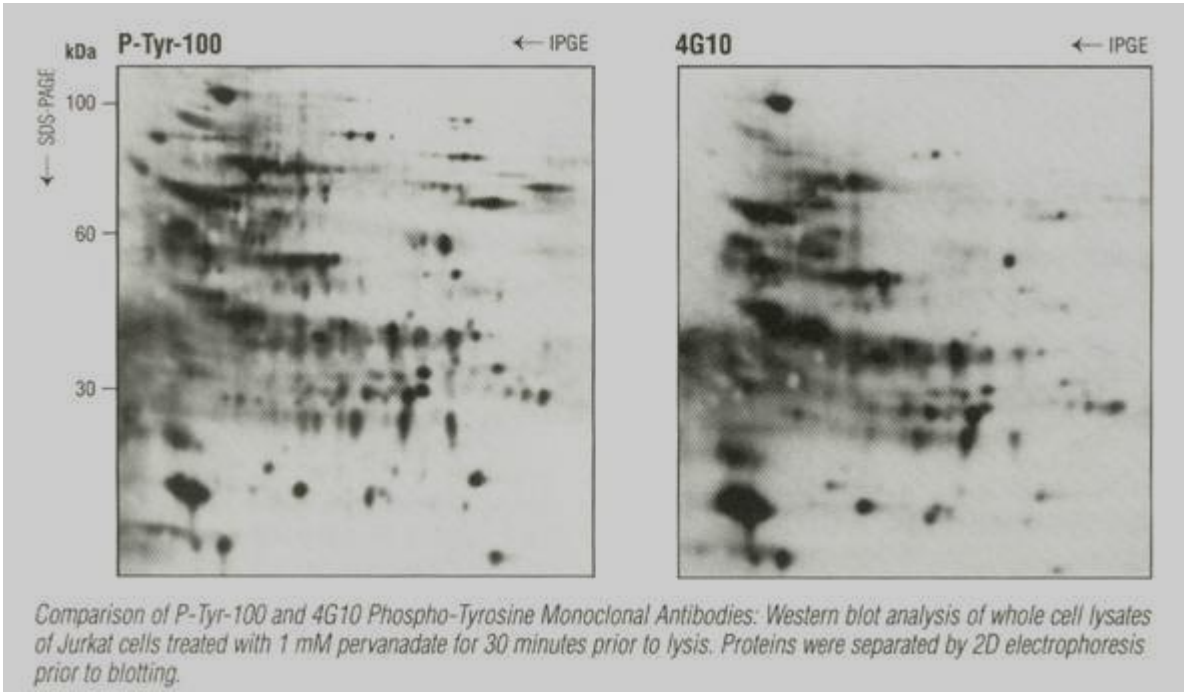


7. Identification of pathway-involving proteins by specific Abs

Phospho-specific antibodies ------P-Tyr Ab
P-Thr Ab
etc.

----Tyr----------
|
PO_4



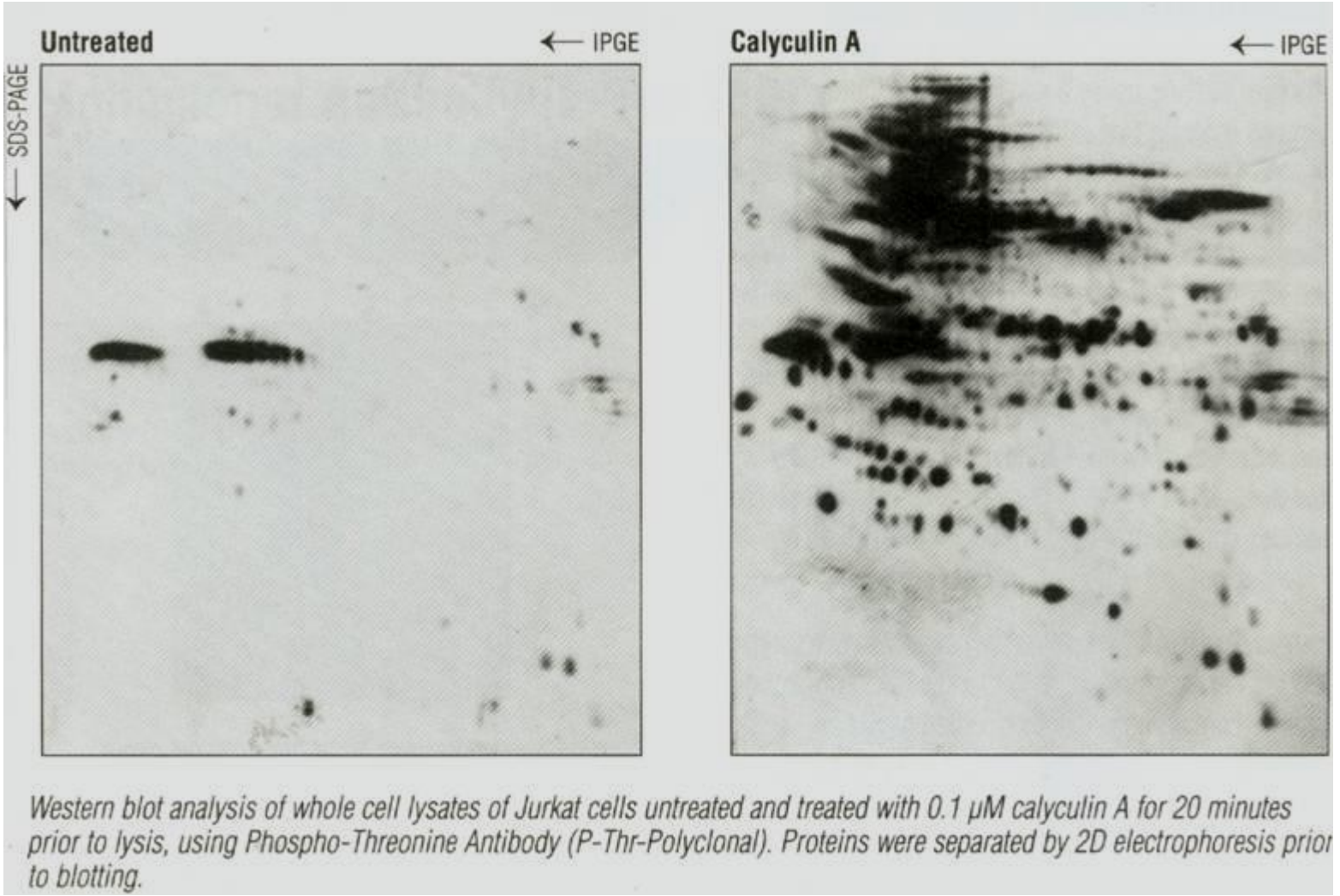


Comparison of P-Tyr-100 and 4G10 Phospho-Tyrosine Monoclonal Antibodies: Western blot analysis of whole cell lysates of Jurkat cells treated with 1 mM pervanadate for 30 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.

(Cell Signaling Tech.)

Phospho-specific antibodies ------ P-Tyr Ab
P-Thr Ab
etc.

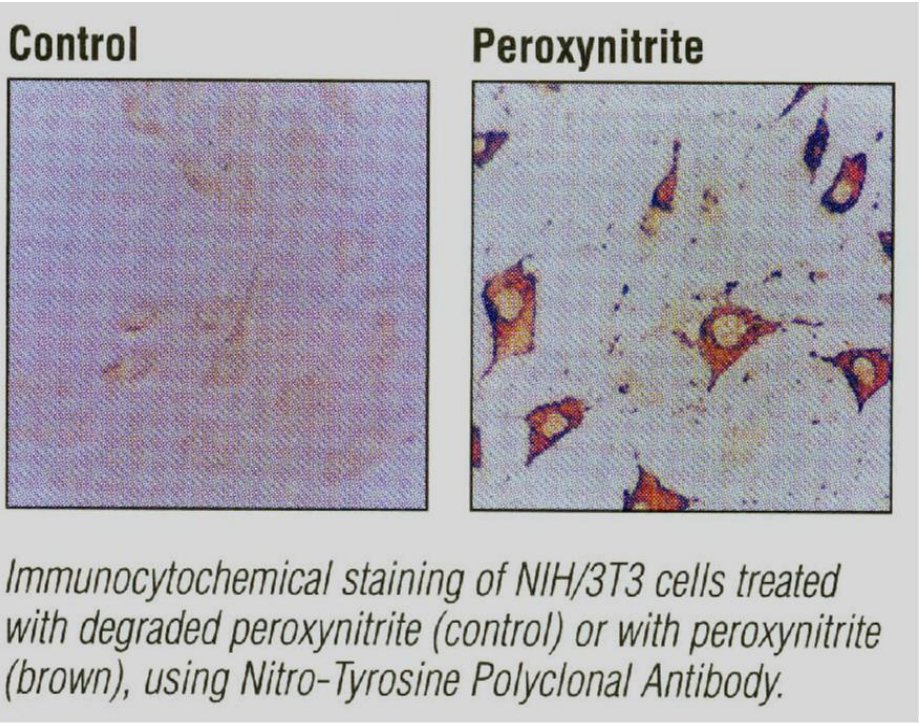
----Thr----------
|
PO_4



Western blot analysis of whole cell lysates of Jurkat cells untreated and treated with 0.1 μM calyculin A for 20 minutes prior to lysis, using Phospho-Threonine Antibody (P-Thr-Polyclonal). Proteins were separated by 2D electrophoresis prior to blotting.

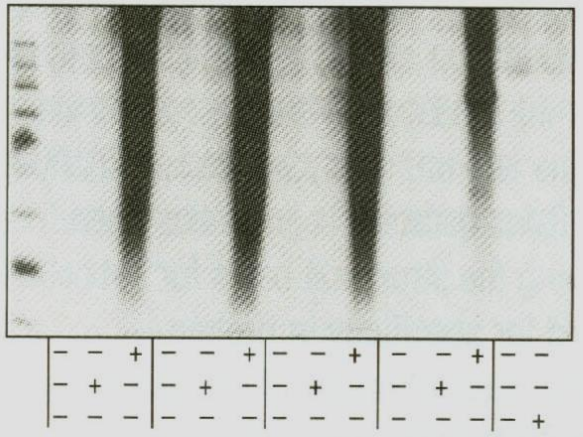
Nitro-Tyrosine specific antibodies --- NO pathway

----Tyr----------
|
NO



Immunocytochemical staining of NIH/3T3 cells treated with degraded peroxynitrite (control) or with peroxynitrite (brown), using Nitro-Tyrosine Polyclonal Antibody.

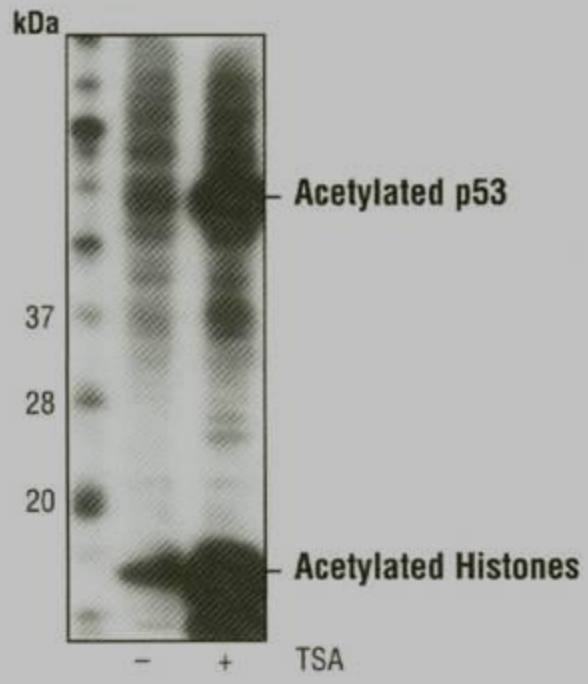
3T3 C6 A431 HeLa 3T3



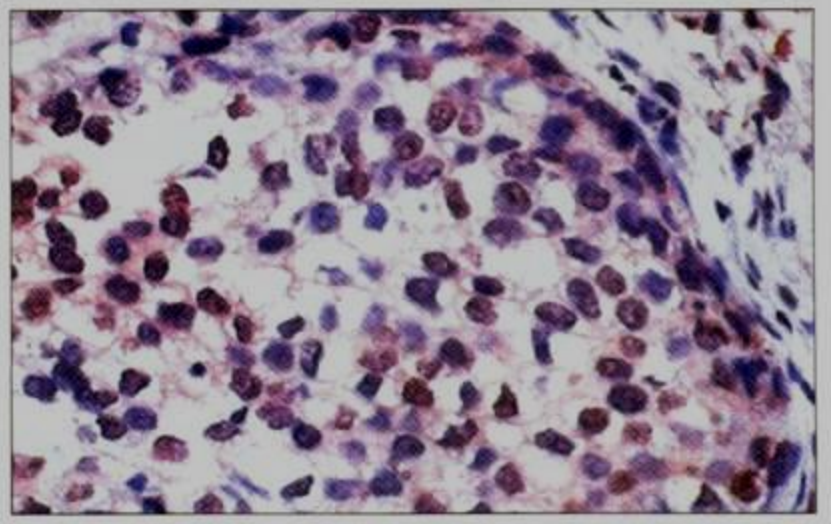
Western blot analysis of whole cell lysates of various cells untreated or treated with peroxynitrite, degraded peroxynitrite or pervanadate, using Phospho-Tyrosine Monoclonal Antibody (P-Tyr-100) #9411 (upper) and Nitro-Tyrosine Polyclonal Antibody (lower).

Acetylated-Lysine specific antibodies --- Acetylation pathway

----Lys--------
|
C_2H_5



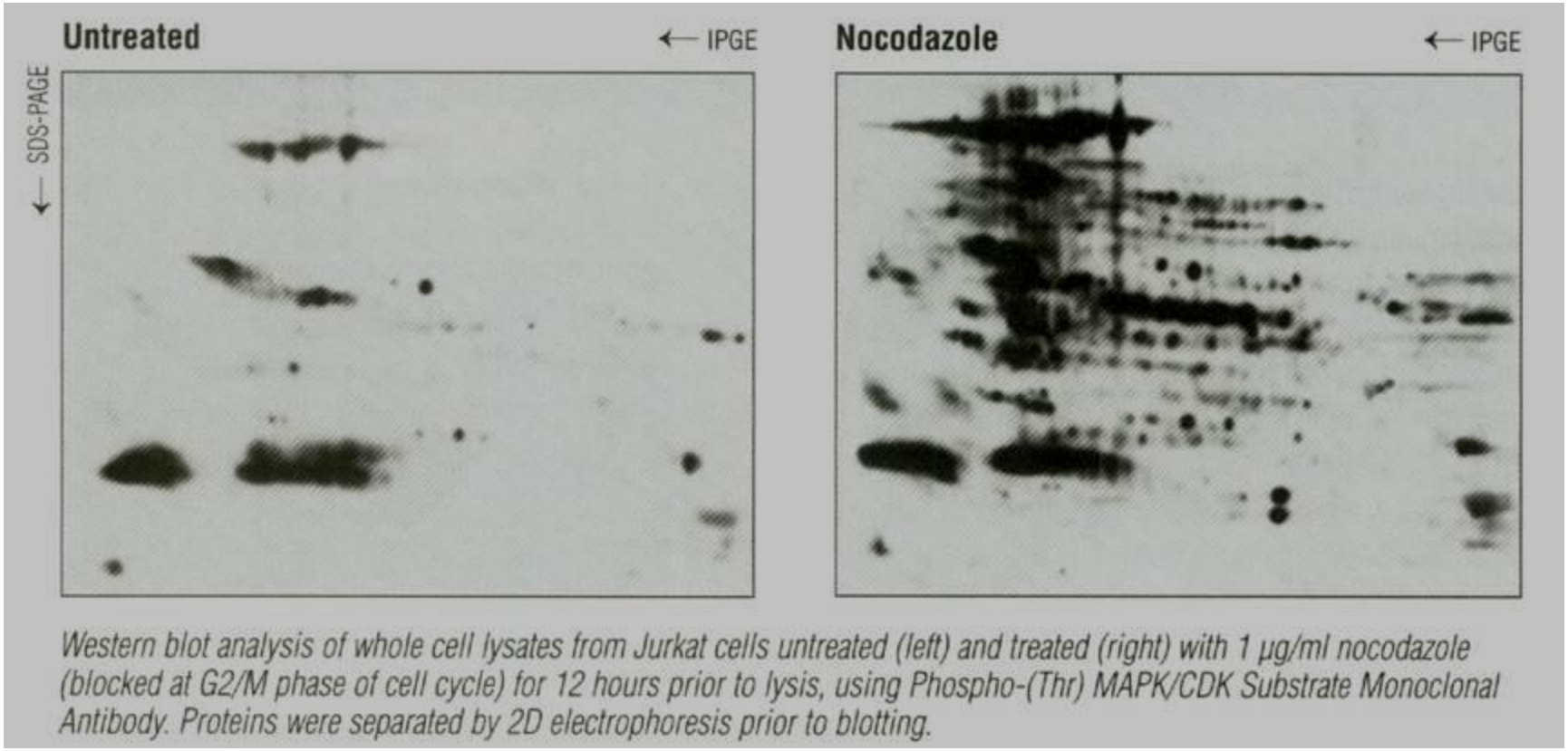
Western blot analysis of COS cell extracts untreated or treated with 0.4 µM TSA for 18 hours, using Acetylated-Lysine Monoclonal Antibody (Ac-K-103).



Nuclear and slight cytoplasmic staining of proteins with acetylated lysine residues in paraffin-embedded human breast tumor section, using Acetylated-Lysine Monoclonal Antibody (Ac-K-103).

Phospho-(Thr) MAPK/CDK Substrate Antibody --- MAPK/CDK pathway

----Thr-Pro-------
|
PO_4



Western blot analysis of whole cell lysates from Jurkat cells untreated (left) and treated (right) with 1 µg/ml nocodazole (blocked at G2/M phase of cell cycle) for 12 hours prior to lysis, using Phospho-(Thr) MAPK/CDK Substrate Monoclonal Antibody. Proteins were separated by 2D electrophoresis prior to blotting.

Another example of the use of antibodies

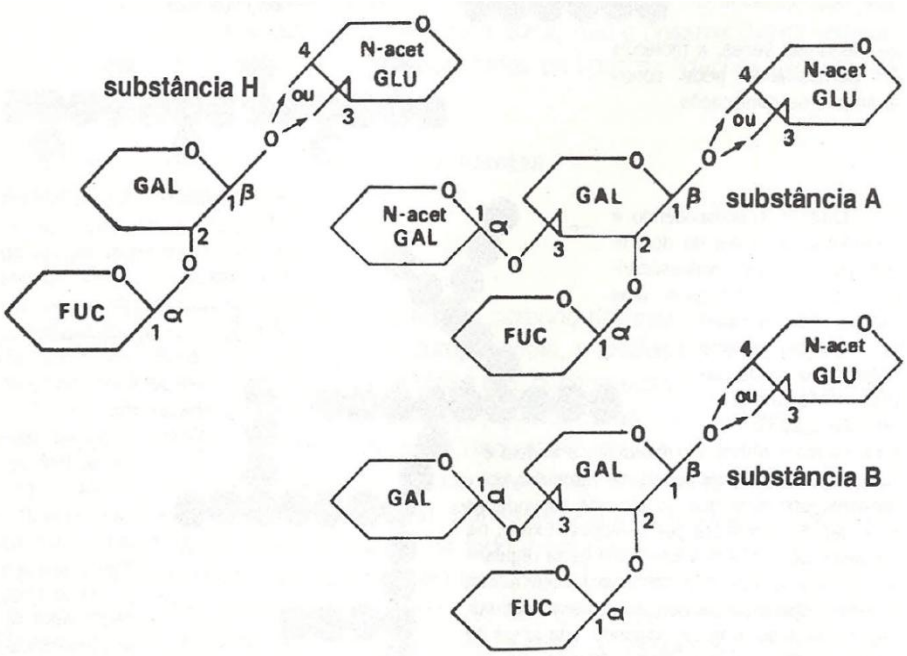
7- BLOOD TYPING

CHARACTERISTIC BLOOD GROUP OLIGOSACCHARIDES

In Human red blood cells, the oligosaccharides responsible for the four major blood groups, 0, A, B, and AB System (ABH (0)) have been intensively studied, isolated and identified.

A- The group 0 red blood cells contain a trisaccharide formed by L-fucose, D-galactose and N-acetyl-D-glucosamine (designated by substance H).
B- The type A red blood cells contain the substance A, which is a tetrasaccharide formed by substance H and N-acetyl-D-galactosamine.
C- Red blood cells of type B contain the tetrasaccharide formed by substance H and D-galactose (substance B).
D- The AB type red blood cells contain both substances A and B.
These oligosaccharides bind glycosphingolipids of the red blood cell membrane by N-acetyl-D-glucosamine.

Besides the system ABH (0), other blood groups have been described, for example, the groups HS and MN, which are associated with other oligosaccharides membrane surface of the erythrocyte. The system MN, the most important after the system ABH (0) in man is due to the antigenic action of the glycoprotein glycophorin, abundant on the red blood cell membrane.



Oligosaccharides determining the antigenic action of human blood group ABH (0). FUC: L-fucose, Gal: D-galactose, N-acetyl GLU: N-acetyl-D-glucosamine, N-acetyl LAG: N-Acetyl-D-galactosamine.

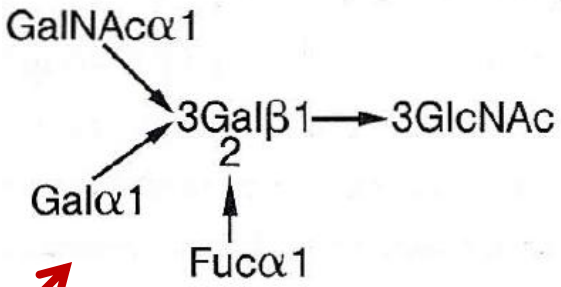
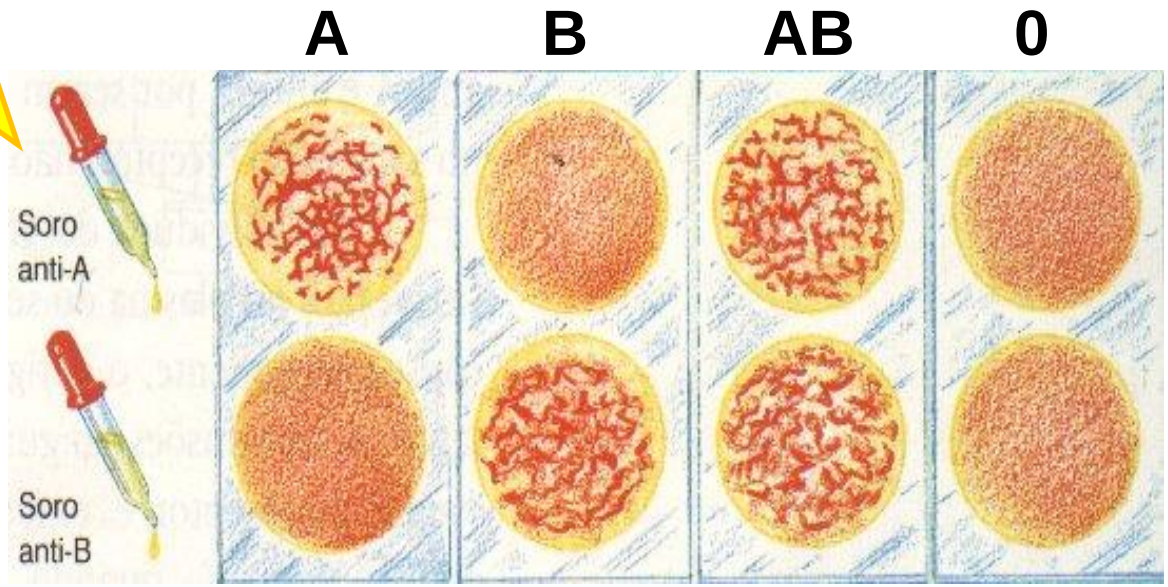


Illustration of the linkage pattern in ABH(0) histo-blood group tri- and tetrasaccharides. The core H(O)-trisaccharide (type I: α-1,2-fucosylated Gal*p*β1-3GlcNA*cp*), whose **L**-fucose part is freely accessible to the eel lectin, can be extended in α-l,3-linkage by either *N*-acetyl-**D**-galactosamine (A epitope) or galactose (B epitope). A branched structure is generated.

How do we find our blood type?

antibodies



Why is it important to know?

--- Blood transfusion

--- Organ transplant

--- Pregnant woman

- Paternity test
- Crime scene analysis

Blood compability