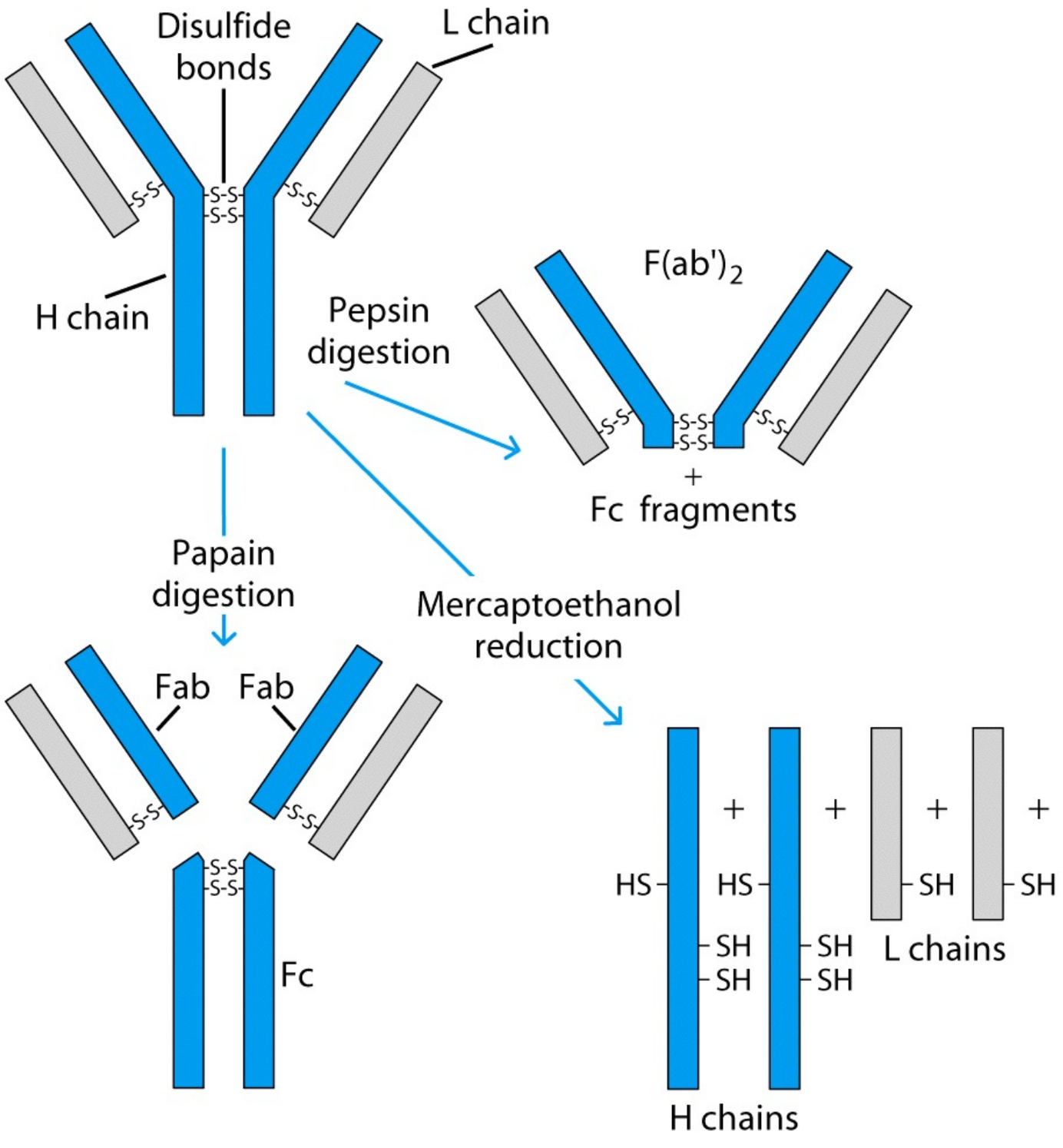
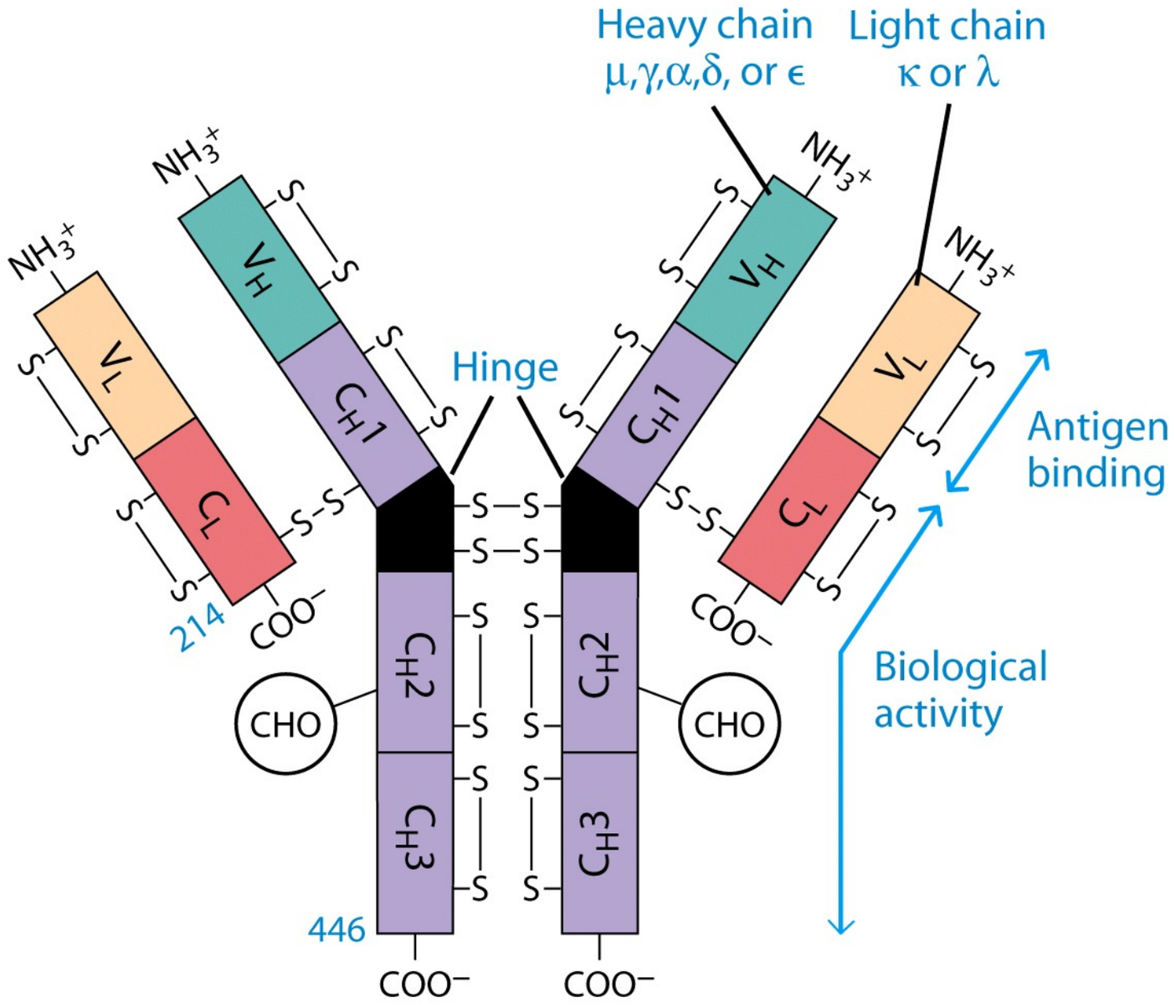
Antibody enzymatic digestion



Antibody structure



Why do antibodies need an Fc region?

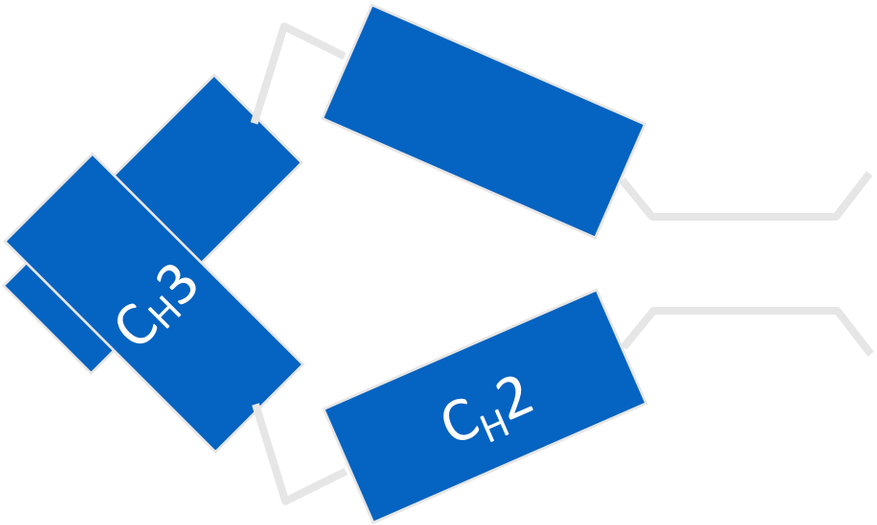
The $(Fab)_2$ fragment can -

- Detect antigen
- Precipitate antigen
- Block the active sites of toxins or pathogen-associated molecules

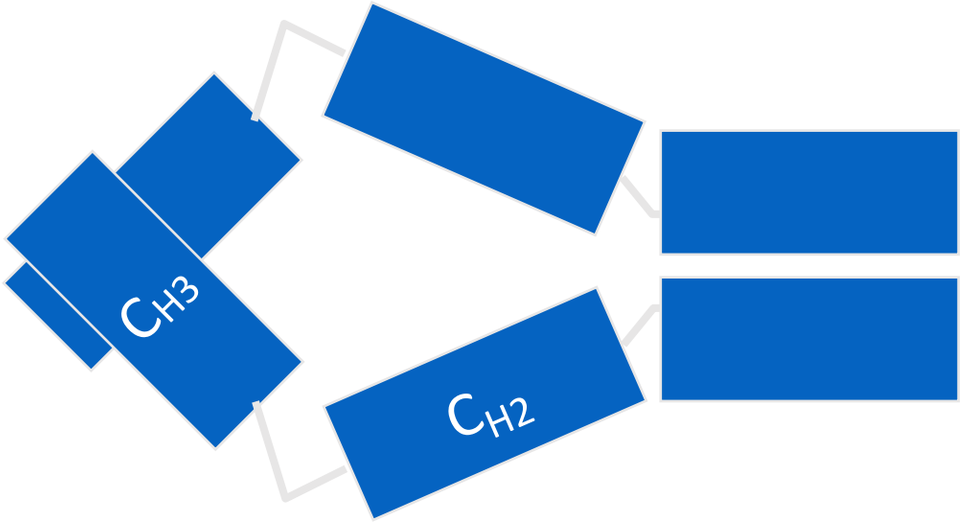
but can not activate

- Inflammatory and effector functions associated with cells
- Inflammatory and effector functions of complement
- The trafficking of antigens into the antigen processing pathways

Structure and function of the Fc region



IgA IgD IgG



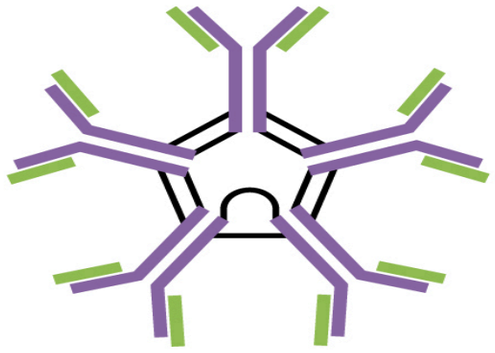
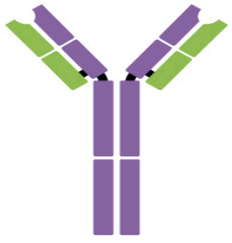
IgE IgM

The hinge region is replaced by an additional Ig domain

Fc structure is common to all specificities of antibody within an ISOTYPE

The structure acts as a receptor for complement proteins and a ligand for cellular binding sites

Antibody Classes And Biological Activities

The Five Immunoglobulin (Ig) Classes					
	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
					
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	no	yes	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor

- When Ag – Ab reactions occur invitro, they are known as serological reactions.

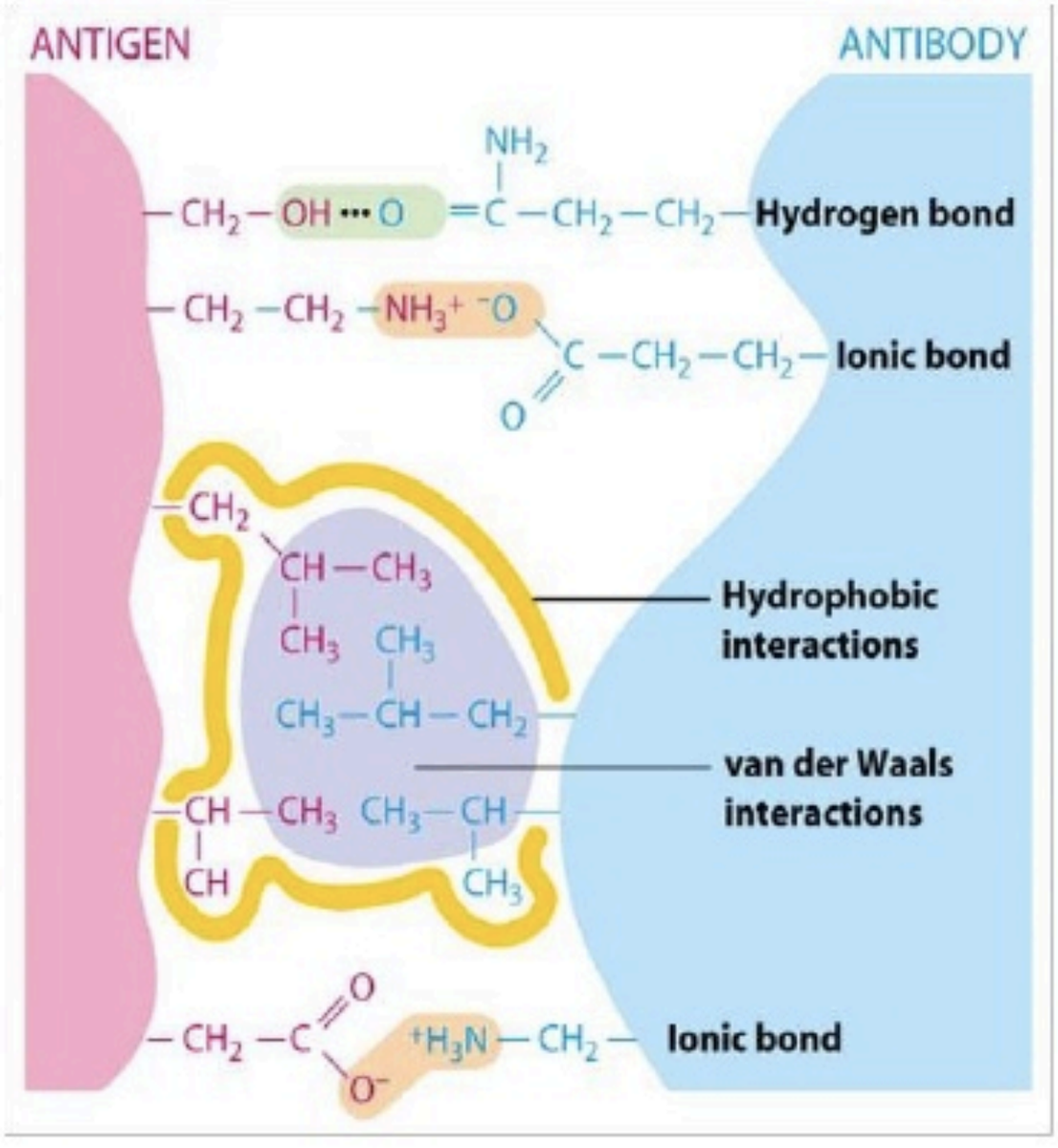
- The reactions between Ag and Ab occur in three stages.
  - In first stage the reaction involves formation of Ag-Ab complex.
  - The second stage leads to visible events like precipitation, agglutination etc.
  - The third stage includes destruction of Ag or its neutralization

Salient Features of Antigen – Antibody Reaction:

- **Specificity of Antigen – Antibody Reaction.**
- **Immune complex.**
- **Binding Site of Antigen – Antibody Reaction.**
- **Binding Force of Antigen – Antibody Reaction.**

Strength of Antigen – Antibody reaction:

•The non – covalent interaction that form the basis of antigen – antibody binding include hydrogen bond, ionic bond, hydrophobic interaction and Van der Waals interaction.



•A strong antigen – antibody interaction depends on a very close fit between the antigen and antibody which requires high degree of specificity.

Properties of Antigen – Antibody Reaction:

The properties of antigen and antibody can be explained with the help of three points. They are:

- **Antibody Affinity.**
- **Antibody Avidity**
- **Cross reaction.**

$$\text{Affinity } K = \frac{[\text{Ab - Ag}]}{[\text{Ab}]\quad[\text{Ag}]} = 10^4 \text{ to } 10^{12} \text{ L/mol}$$

Avidity of Antibody:

- It is the strength of the bond after the formation of Ag-Ab complexes.
- It is used to denote the overall capacity of antibodies to combine with the multivalent antigen.
- A multivalent Ag has many types of antigenic determinants.
- When injected into the blood, each antigenic determinant stimulates the production of a particular antibody.

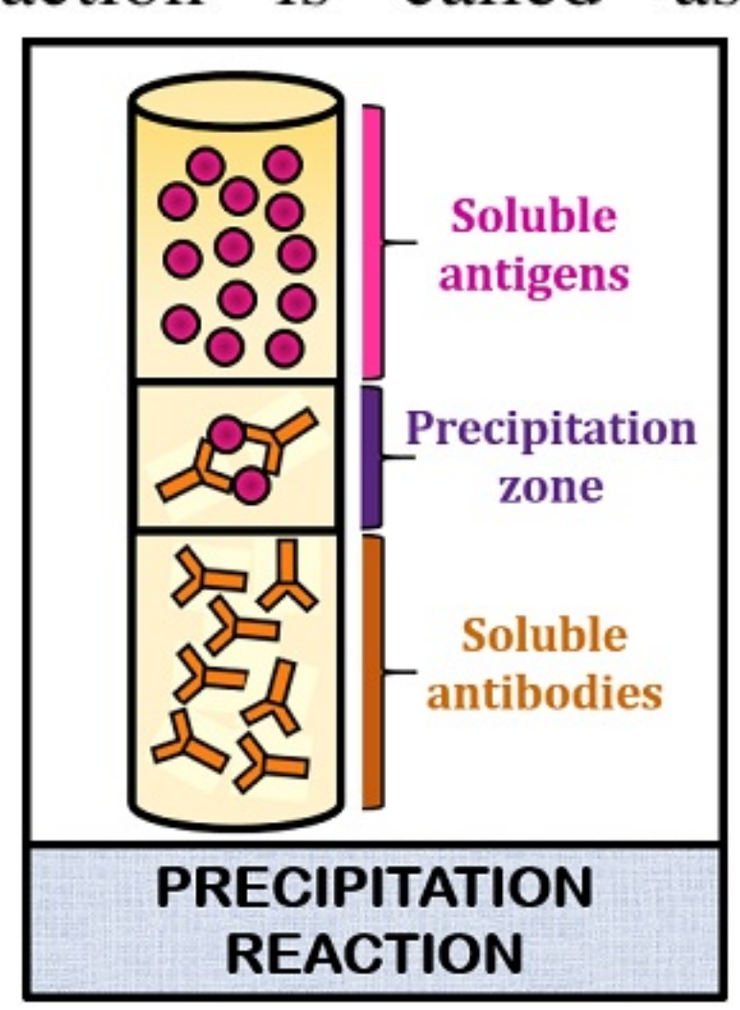
Types of Antigen – Antibody Reaction:

The types of antigen – antibody reactions are:

- **Precipitation Reaction.**
- **Agglutination Reaction.**
- **Complement Fixation.**
- **ELISA – Enzyme Linked ImmunoSorbent Assay.**

Precipitation Reaction:

When a soluble Ag combines with its Ab in the presence of an electrolyte (NaCl) at a particular temperature and pH, it forms an insoluble precipitate of Ag-Ab complex. The Ab causing precipitation is called Precipitin and the reaction is called as precipitation reaction.

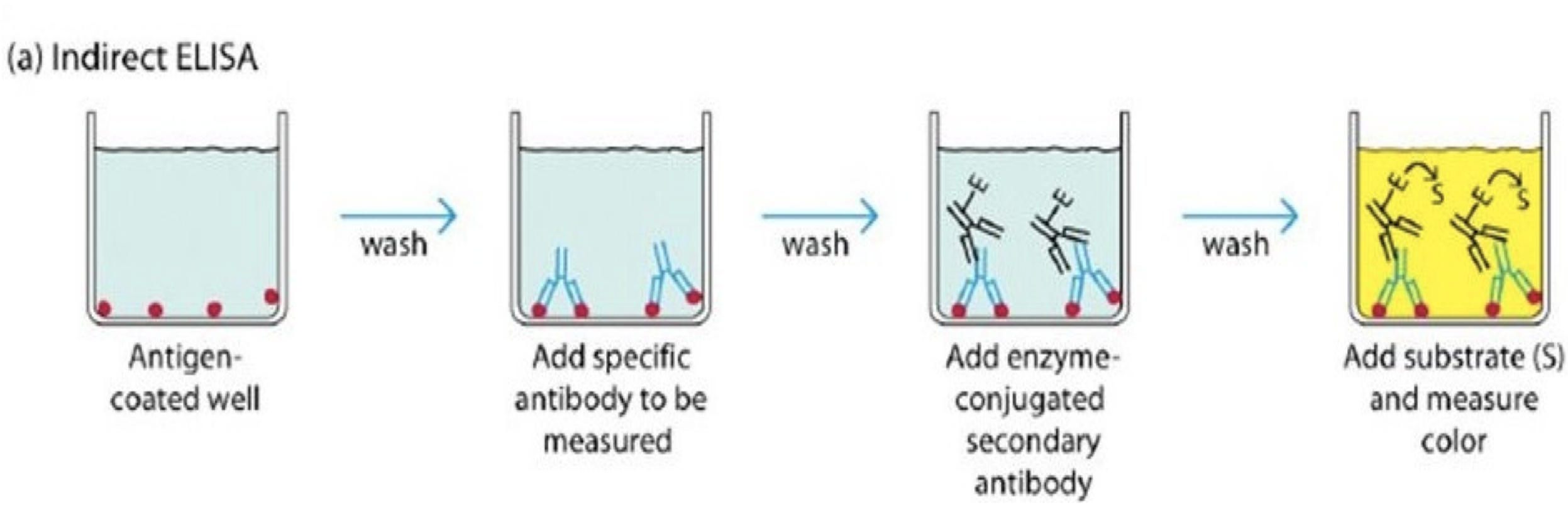


Agglutination Reaction:

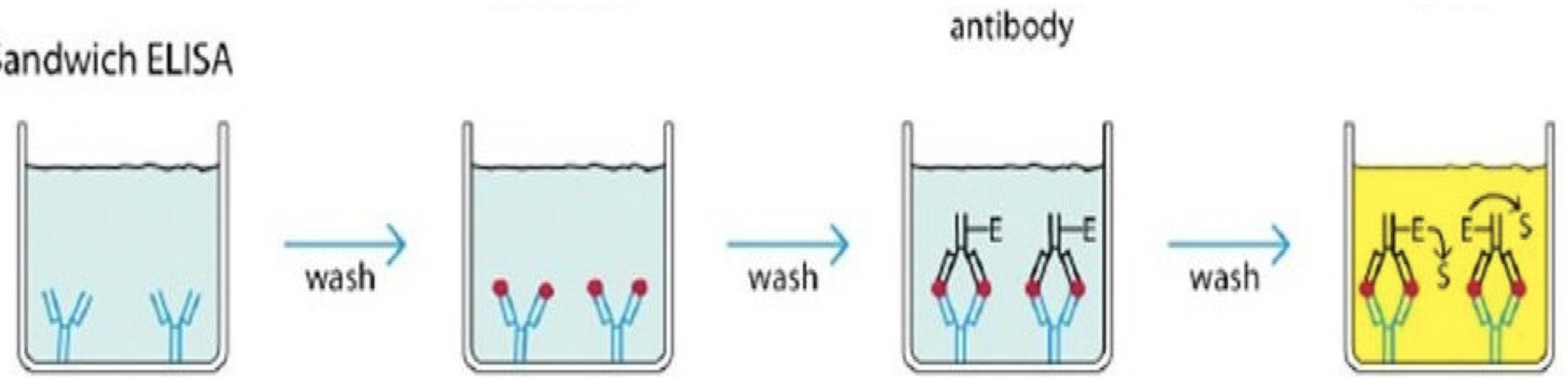
• When a particular Ag is mixed with its Ab's in the presence of electrolytes at a suitable temperature and pH, the particles are clumped or agglutinated.

• The Ab of the serum causes the cellular Ag's to form clumps and these are called Agglutinins.

ELISA (enzyme-linked immunosorbent assay)



(b) Sandwich ELISA



Antibody-coated well

Add antigen to be measured

Add enzyme-conjugated secondary antibody

Add substrate and measure color

Epitopes

4
1
Antigen
3
2

Isolate spleen cells

Plasma cells

+

Myeloma cells

Hybridize

Hybridomas

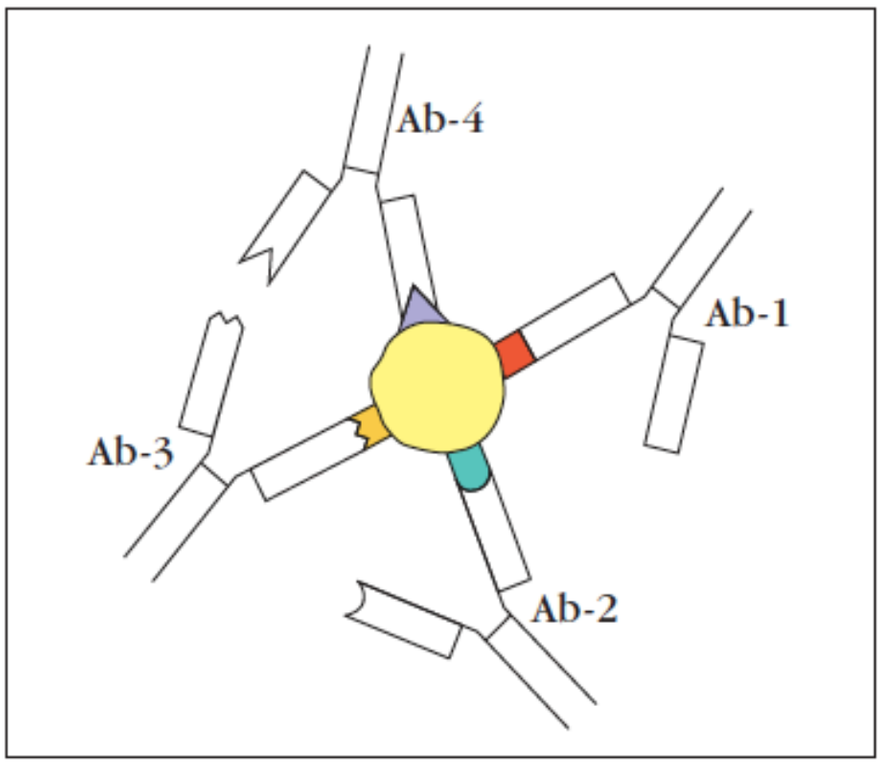
Select

Clones

Isolate serum

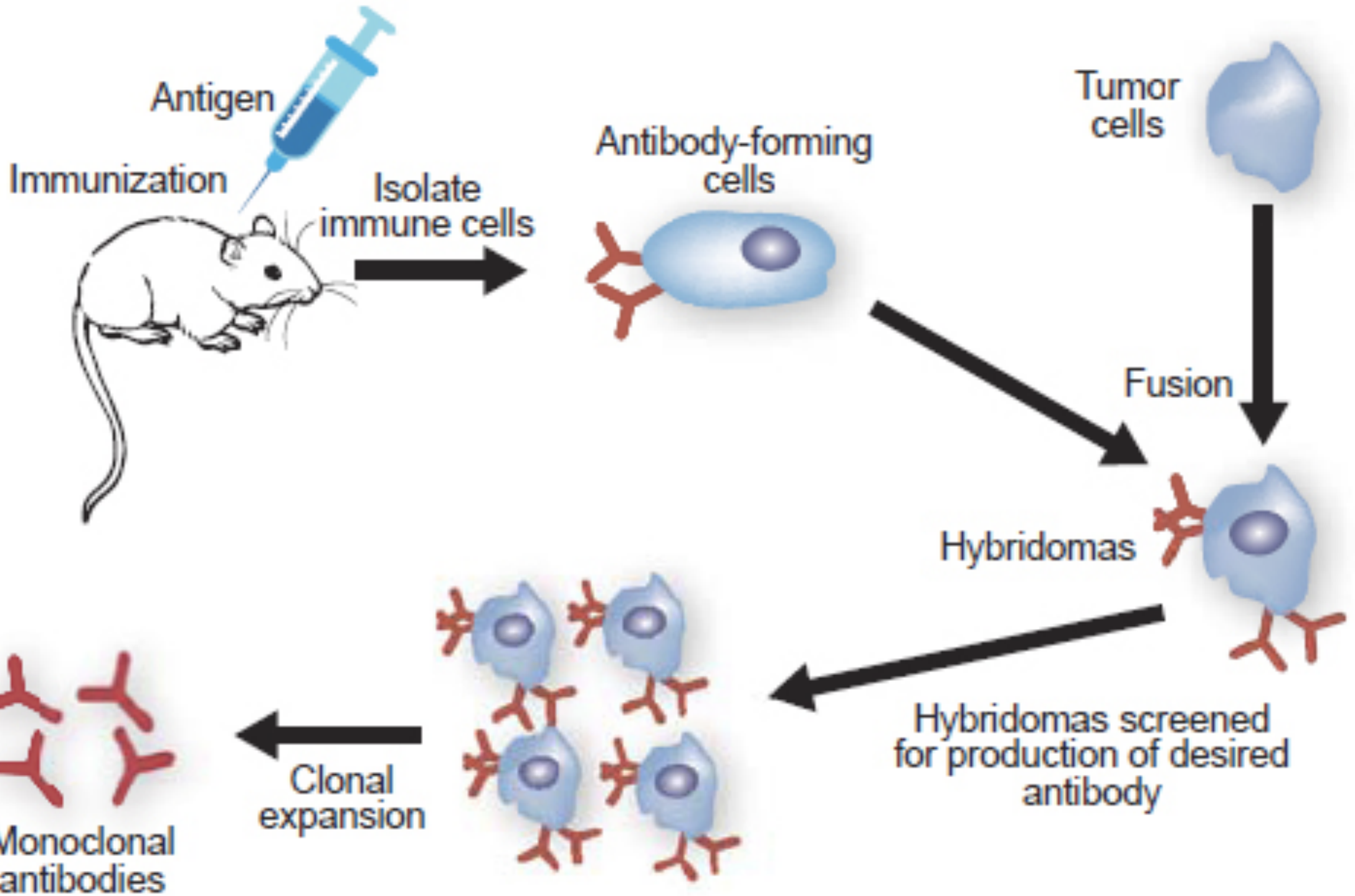
Ab-1
Ab-2
Ab-3
Ab-4

Polyclonal antiserum



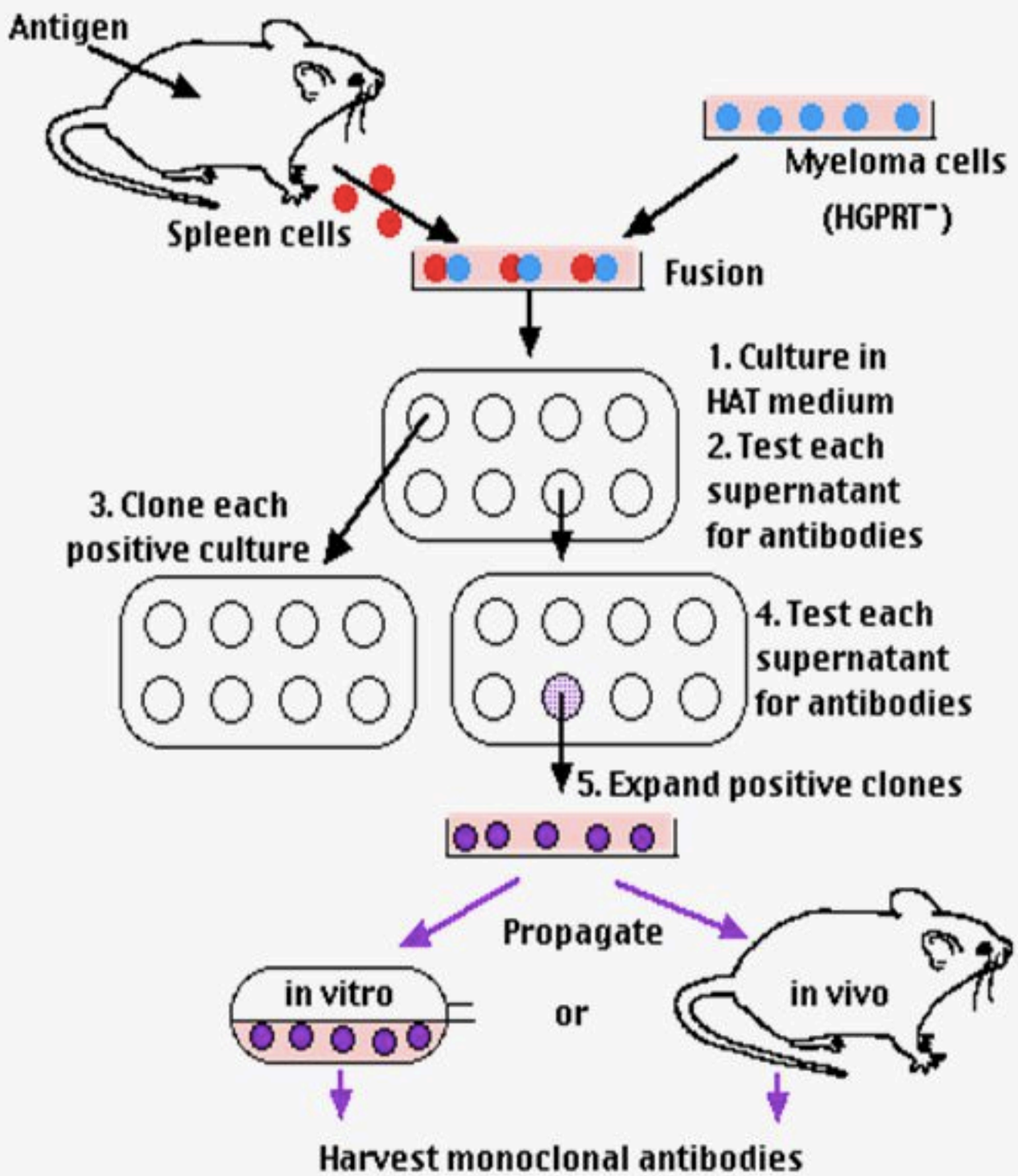
Ab-1
Ab-2
Ab-3
Ab-4

Monoclonal antibodies


Antigen
Immunization
Isolate immune cells
Antibody-forming cells
Tumor cells
Fusion
Hybridomas
Hybridomas screened for production of desired antibody
Clonal expansion
Monoclonal antibodies

HYBRIDOMA TECHNOLOGY

- 1) Immunize animal (mouse or rabbit)
- 2) Isolate spleen cells (containing antibody-producing B cells)
- 3) Fuse spleen cells with myeloma cells (e.g. using PEG - polyethylene glycol)
- 4) Allow unfused B cells to die
- 5) Add HAT culture to kill unfused myeloma cells
- 6) Clone remaining cells (place 1 cell per well and allow each cell to grow into a clone of cells)
- 7) Screen supernatant of each clone for presence of the desired antibody (ELISA)
- 8) Grow the chosen clone of cells in tissue culture indefinitely.
- 9) Harvest antibody from the culture supernatant.



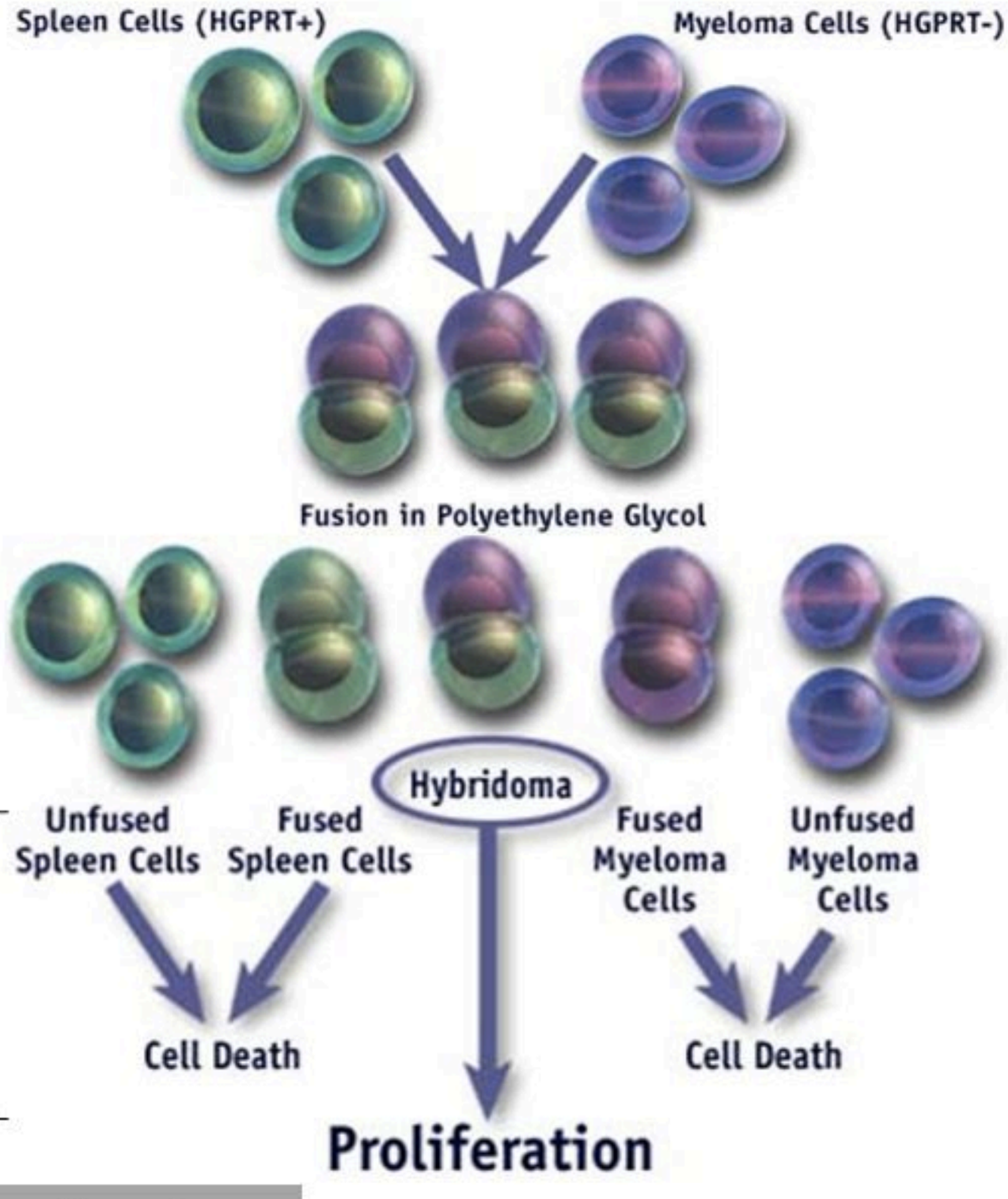
Principle of the HAT selection

Pathways of the nucleic acid synthesis

- Salvage synthesis pathway
  (Needs HGPRT enzyme)
  Mutated in the myeloma cells
- De novo synthesis pathway
  (blocked by the HAT medium)

HAT = hypoxanthine, **aminopterin**, and thymidine

The combination of aminopterin, a drug that acts as a powerful folate metabolism inhibitor, with hyproxanthine and thymidine, which are intermediates in DNA synthesis, provides a form of artificial selection for cells containing functioning hypoxanthine-guanine phosphoribosyltransferase (HGPRT)



HGPRT= hypoxanthine-guanine phosphoribosyltransferase

Trick : Fuse antibody producing B cell (HGPRT +) with myeloma cell (HGRPT-) and select on HAT medium

Immortal
Cannot grow on HAT medium
(HGPRT-)

Limited Life Span
Antibody Prodction
Can grow on HAT medium
(HGPRT+)

Cell Fusion using PEG

Selection on HAT medium

Cannot grow on HAT

Immortal
Antibody Producing

Limited Life Span