Antibody Biology and Biotechnology

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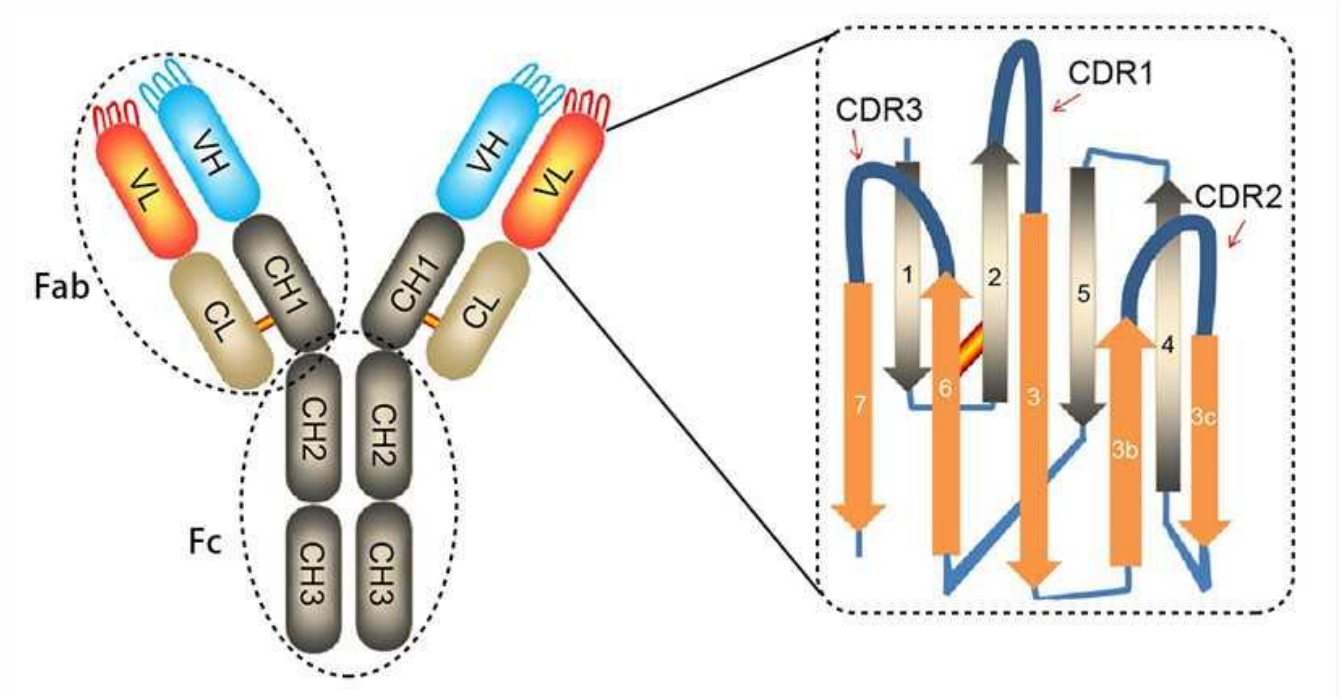
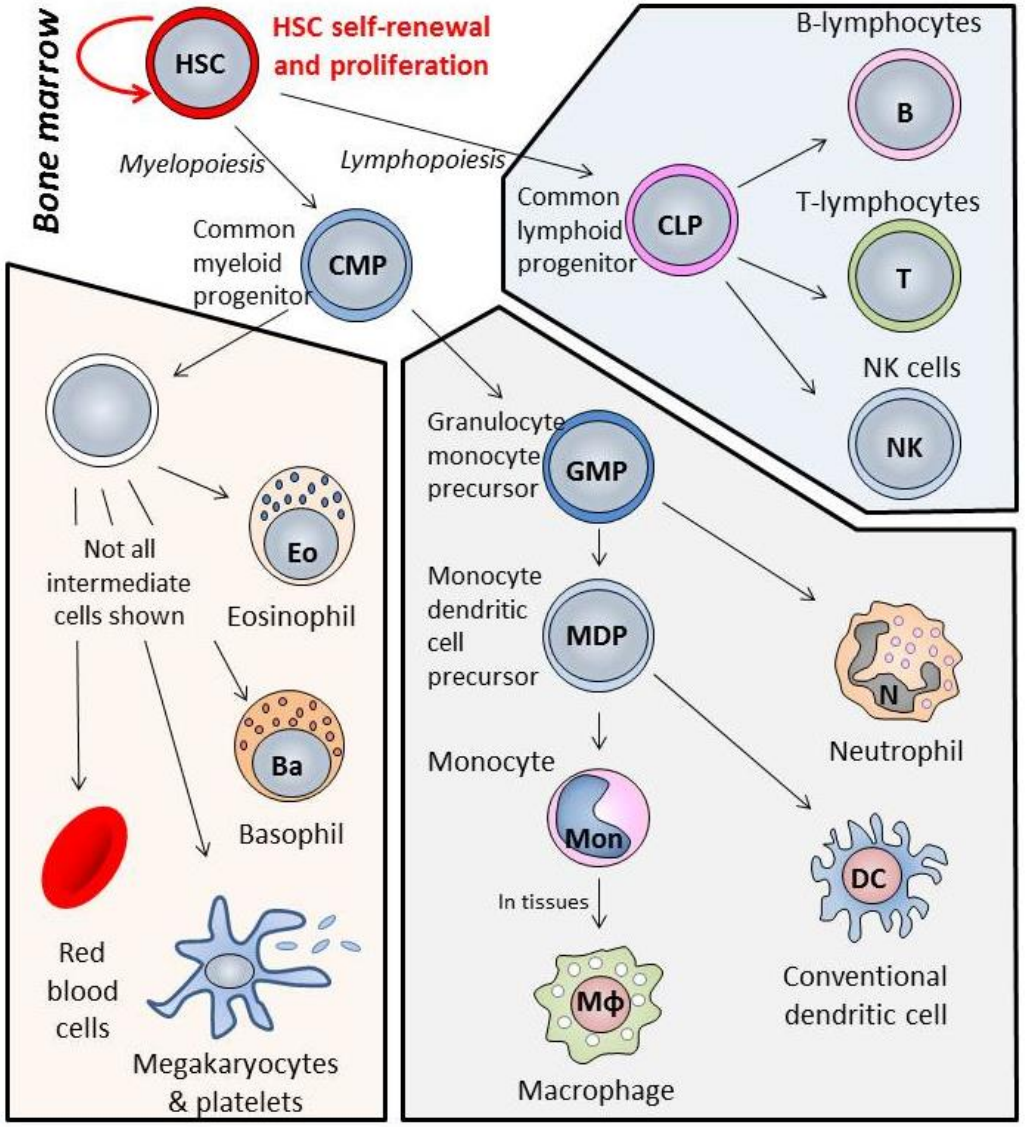
Introduction to Antibodies

- Antibodies are among the most important proteins studied in biotechnology and biomedical science.
- Their ability to recognise specific molecular targets has made them indispensable for research, diagnostics, and medicine.
- Antibodies are also a major component of the adaptive immune system and provide protection against infection and disease.
- An understanding of antibody biology is therefore important both in the laboratory and in the clinic



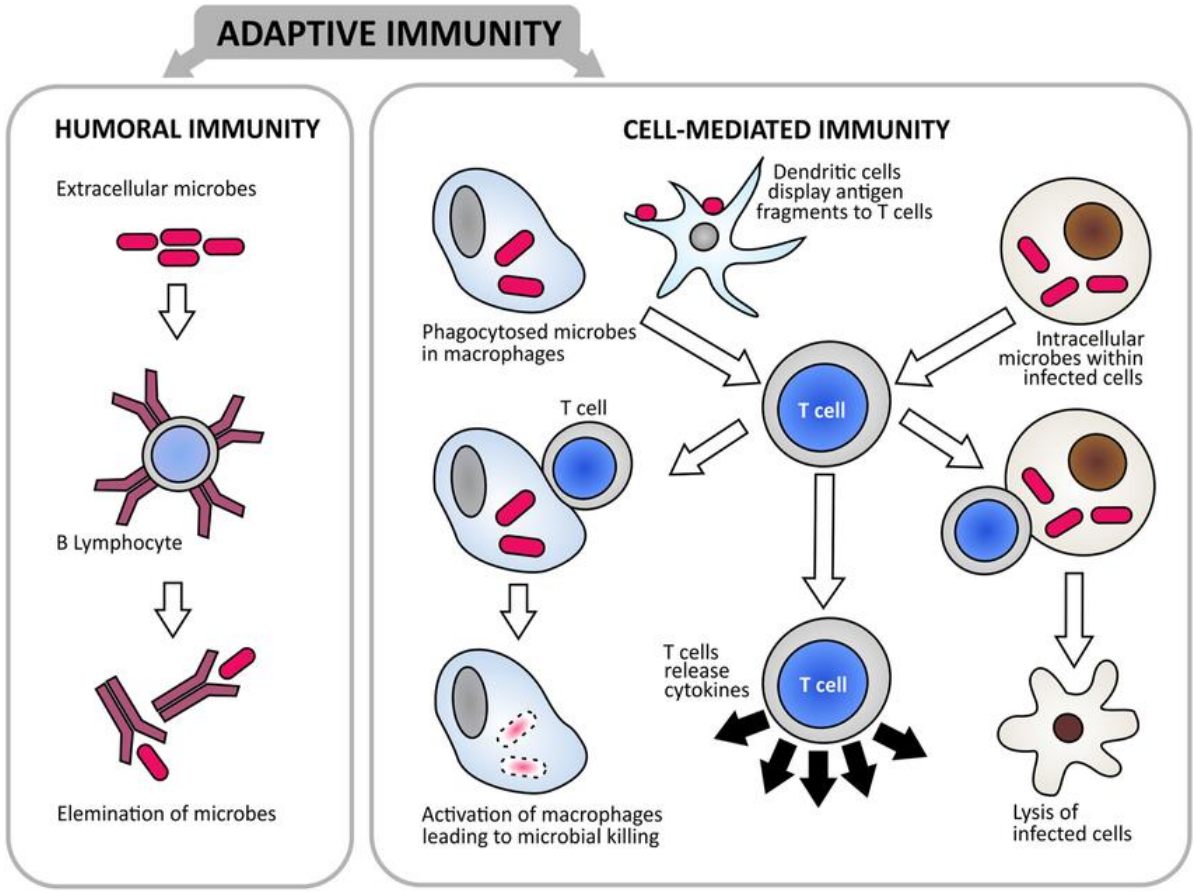
B-Lymphocytes and Antibody Production

- Antibodies are produced by specialised white blood cells known as B-lymphocytes.
- Each B-cell produces an antibody with a unique antigen specificity.
- Following activation, B-cells proliferate and differentiate into plasma cells capable of secreting large quantities of antibody.
- A proportion of activated cells become memory B-cells, allowing a more rapid response if the same antigen is encountered again.



Antibodies in Adaptive Immunity

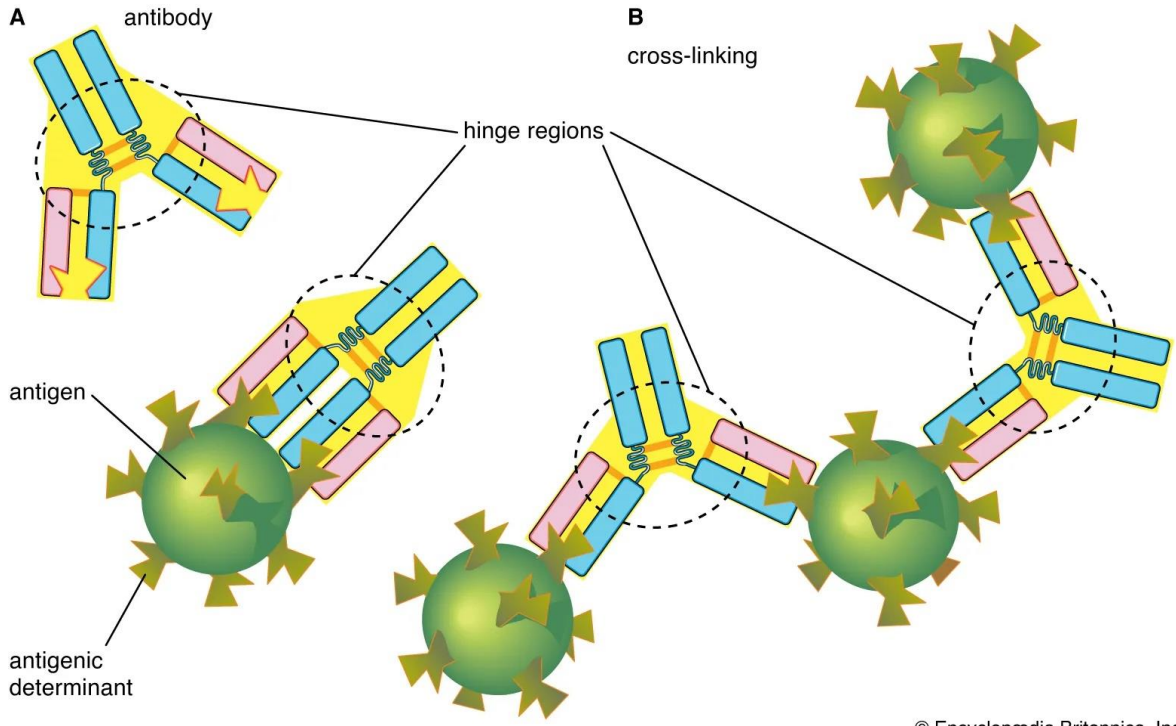
- The adaptive immune system provides highly specific protection against pathogens and other foreign molecules.
- Millions of different B-cell clones circulate within the body, each expressing a unique antibody receptor.
- Collectively, these B-cells provide the capacity to recognise an enormous range of antigens.
- Antibodies contribute to protection by neutralising toxins, preventing pathogen attachment, and promoting elimination by immune cells.



Antigens are the Targets of Antibodies

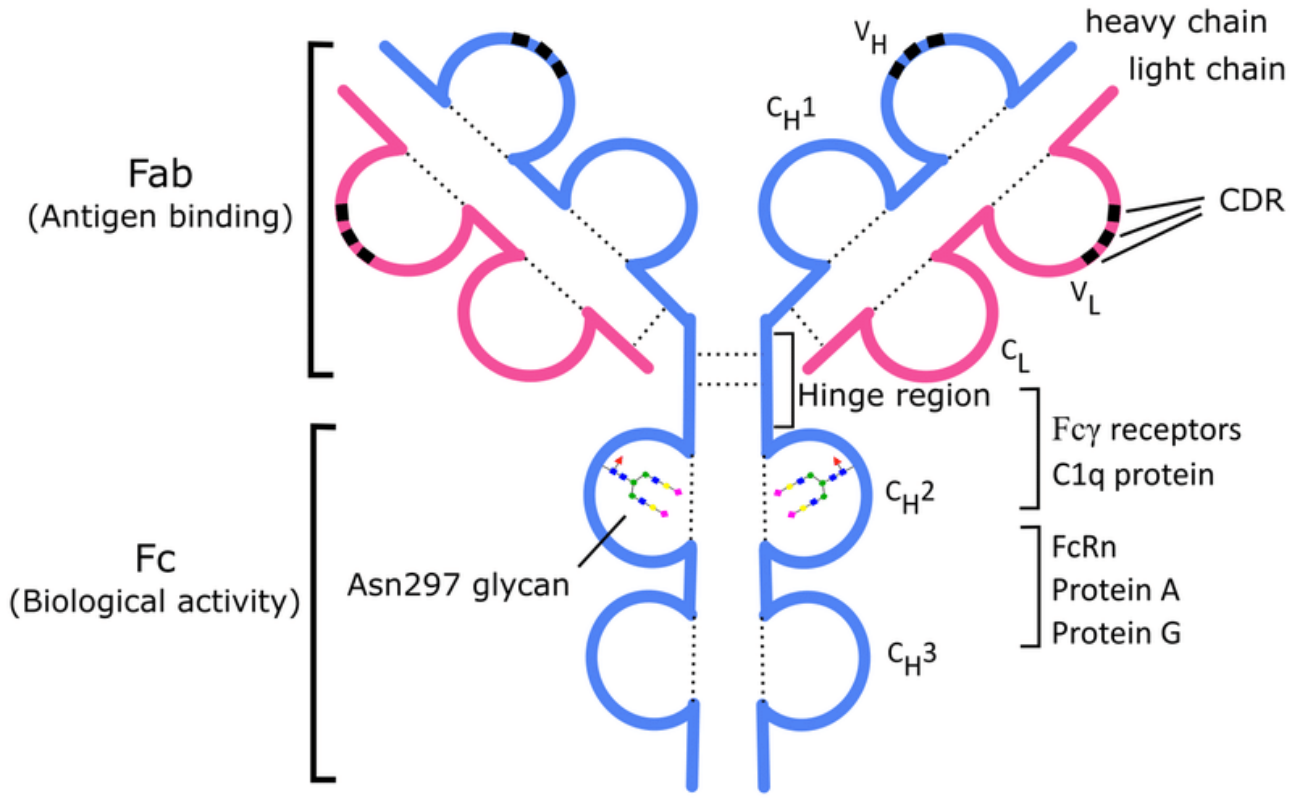
- Antibodies recognise molecules known as antigens.
- Most antigens encountered in research and medicine are proteins, although carbohydrates, lipids, toxins, and other biological molecules may also serve as antibody targets.
- The ability of antibodies to discriminate between closely related molecular structures gives them exceptional specificity.
- This specificity forms the basis of both immune protection

  and many biotechnology applications.



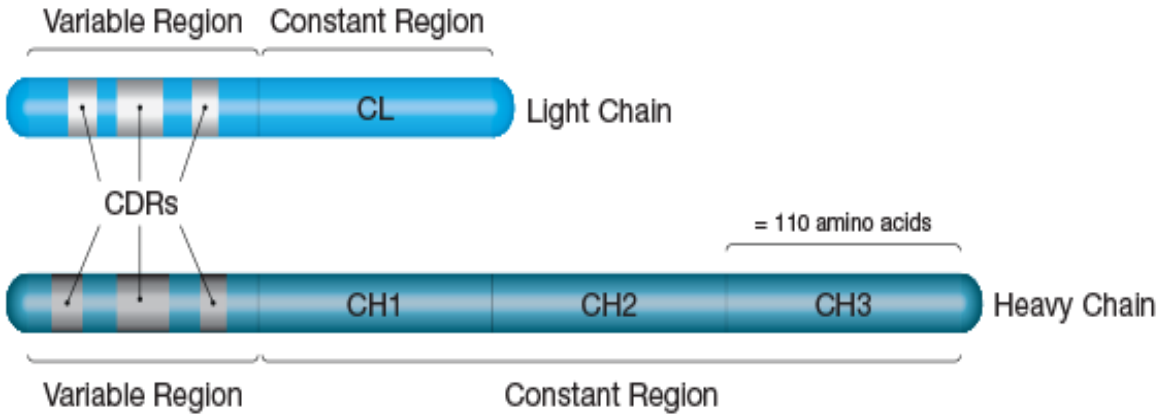
General Structure of Antibody Molecules

- Despite their diversity, antibodies share a common structural organisation.
- Each antibody molecule contains two identical heavy chains and two identical light chains joined together by disulphide bonds.
- The resulting Y-shaped structure contains two antigen-binding sites and a constant region responsible for interactions with the immune system.
- This arrangement allows antibodies to recognise targets while simultaneously recruiting effector mechanisms.



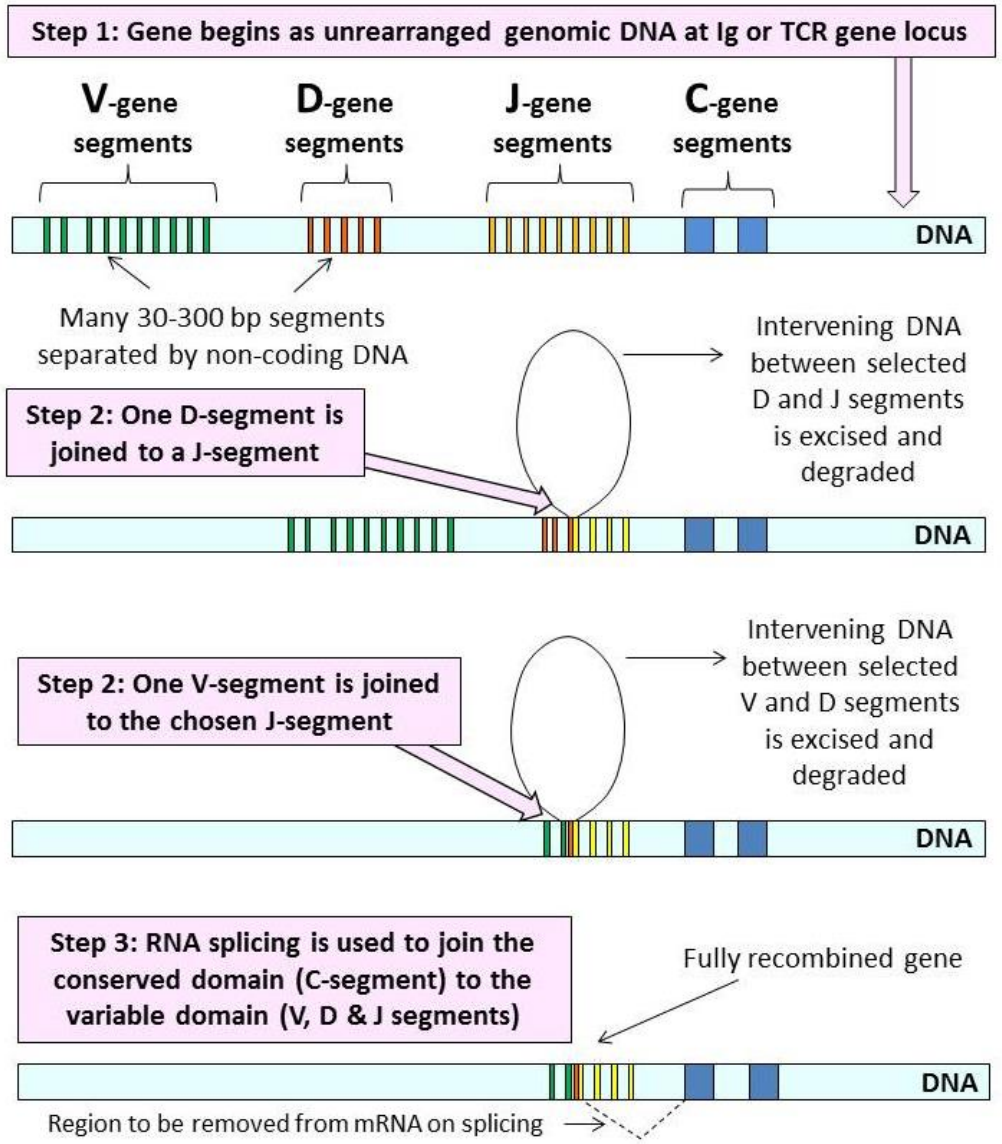
Variable and Constant Regions

- Not all regions of an antibody molecule perform the same function.
- The variable domains located at the tips of the molecule determine antigen specificity.
- The constant domains are more conserved and influence how the antibody interacts with complement proteins and immune cells.
- Different constant regions give rise to different antibody classes, including IgG, IgA, IgM, IgE, and IgD.



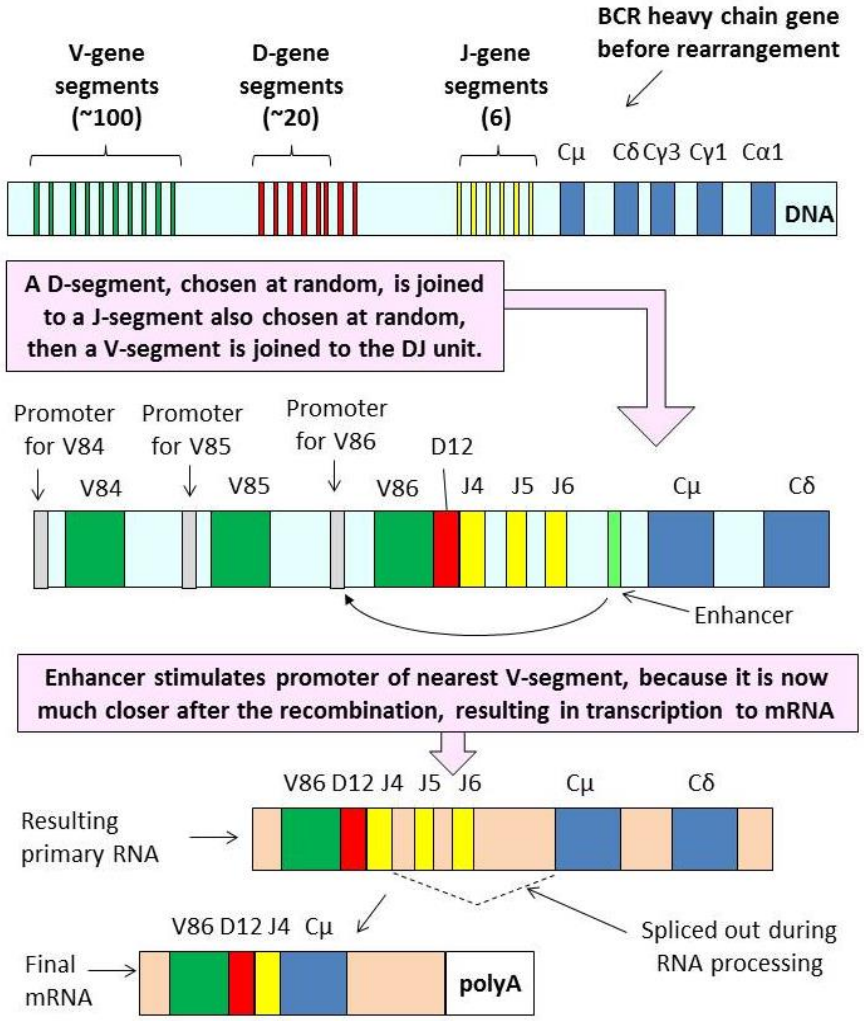
Generation of Antibody Diversity

- The human immune system is capable of producing millions of different antibodies despite possessing far fewer genes than this.
- This diversity arises through genetic rearrangement during B-cell development, where multiple segments of chromosomal DNA are brought together to create functional antibody genes.
- Different combinations of Variable (V), Diversity (D), and Joining (J) gene segments generate a vast range of possible antigen-binding sites.
- As a result, every mature B-cell expresses an antibody with a unique binding specificity.



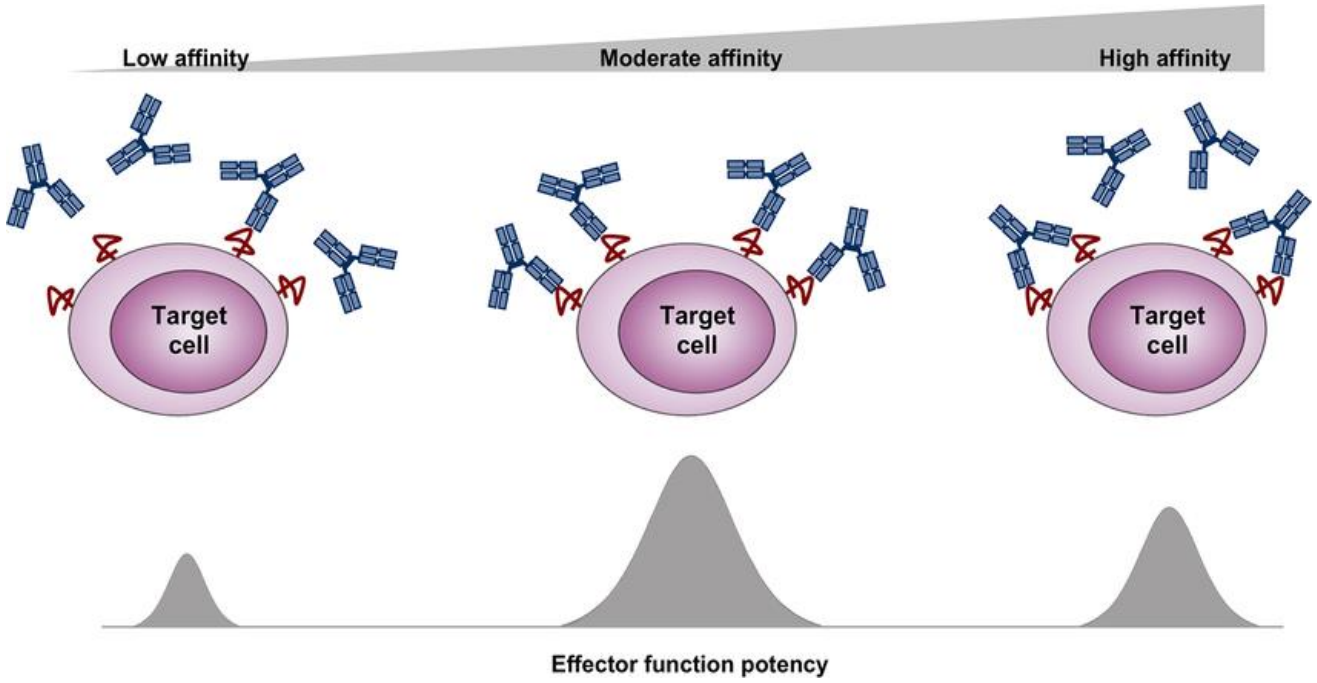
The Human Immunoglobulin Heavy Chain Locus

- The human immunoglobulin heavy chain locus contains approximately 100 Variable (V) gene segments located towards the 5' end of the chromosome.
- Downstream of these are approximately 20 Diversity (D) segments and 6 Joining (J) segments.
- During B-cell development, one V segment, one D segment, and one J segment are selected and joined together to create a functional variable region gene.
- The rearranged variable region is subsequently expressed alongside one of several constant region genes, which determine the antibody isotype produced.



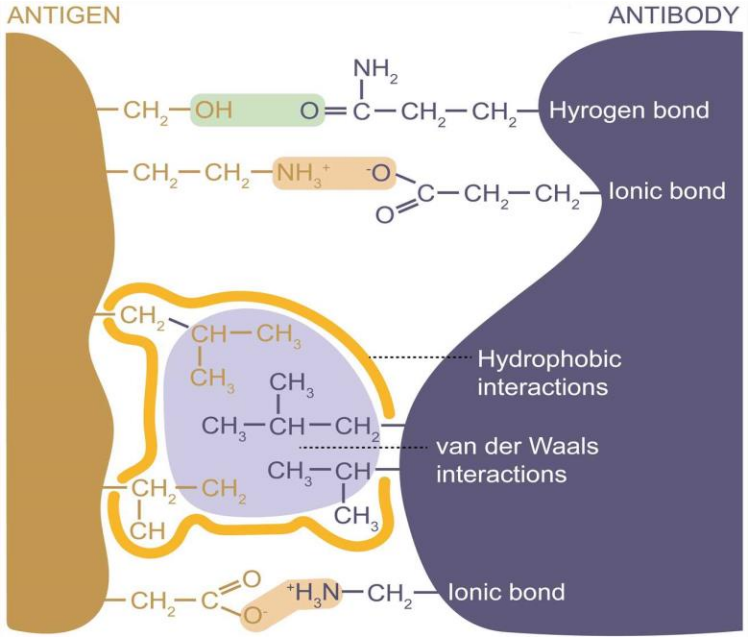
Antibody Affinity and Dissociation Constant (Kd)

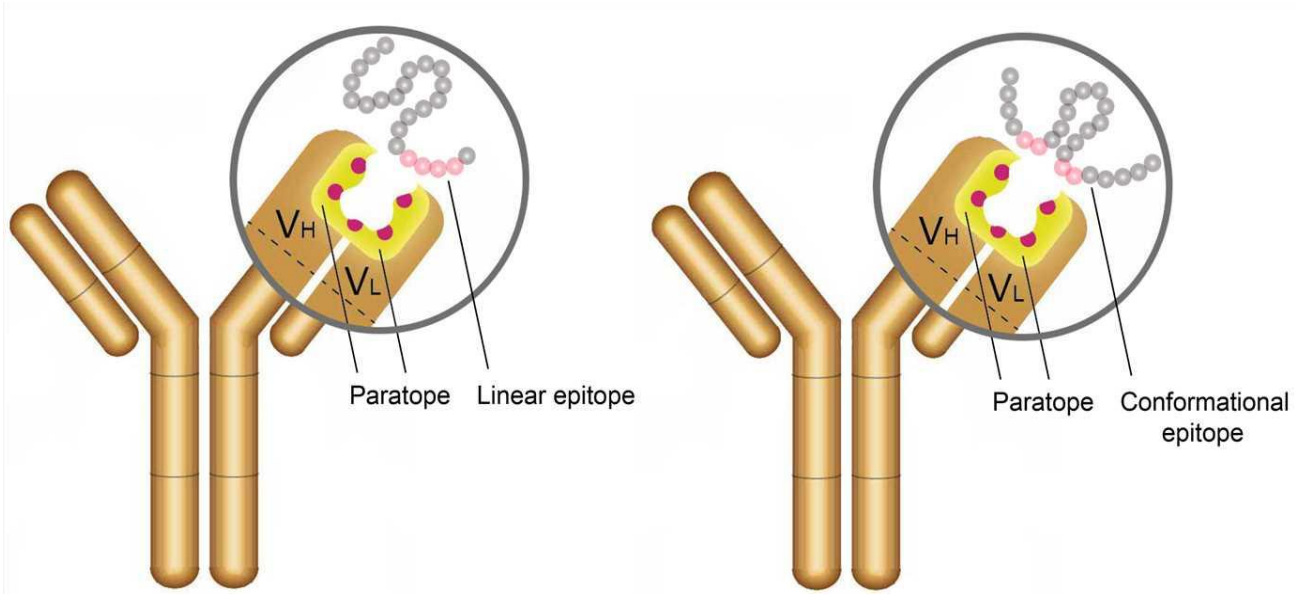
- The strength of interaction between an antibody and its target antigen is known as affinity.
- Affinity is commonly expressed using the dissociation constant (Kd), where lower Kd values indicate stronger binding.
- During the early stages of an immune response, antibodies often bind relatively weakly, with Kd values typically in the range of 10^{-7} to 10^{-9} M.
- Following somatic hypermutation and affinity maturation, antibodies with substantially stronger binding may be generated, with Kd values of 10^{-11} M or lower.



Antibodies Recognise Specific Epitopes

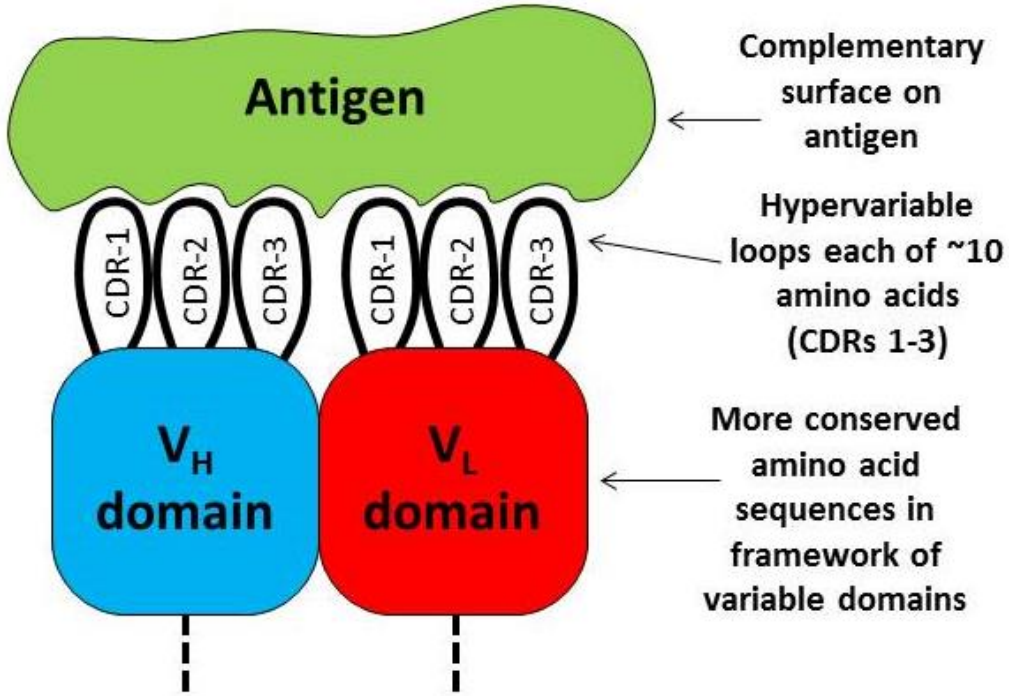
- Antibodies do not normally bind entire antigens.
- Instead, recognition occurs through a small region known as an epitope.
- Some epitopes consist of a continuous stretch of amino acids and are described as linear epitopes.
- Others depend upon the three-dimensional structure of the protein and are known as conformational epitopes.
- The type of epitope recognised can influence how well an antibody performs in different laboratory techniques.





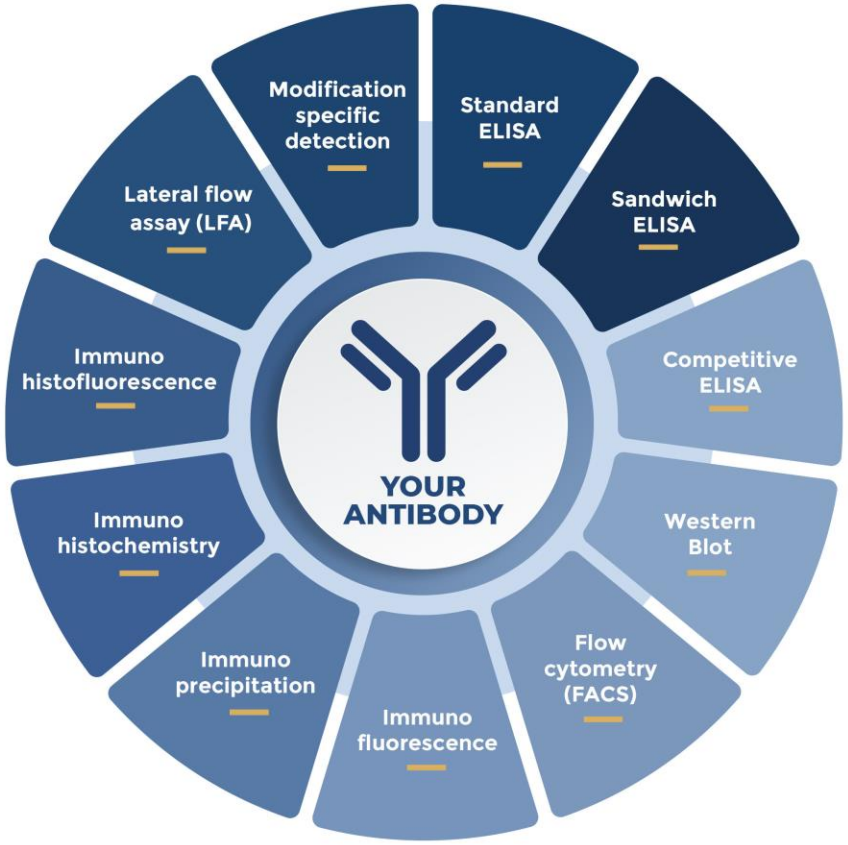
Complementarity Determining Regions

- Most of the contact between an antibody and its target occurs through six short hypervariable loops known as complementarity determining regions (CDRs).
- Three CDRs are contributed by the heavy chain and three by the light chain.
- The sequence of these loops largely determines binding specificity.
- Modern antibody engineering frequently focuses on modification of CDR sequences to improve affinity or alter target recognition.



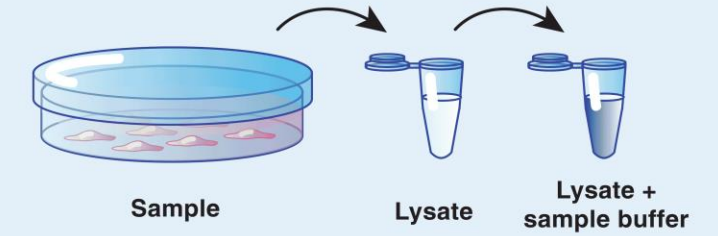
Antibodies are Essential Research Tools

- The specificity of antibody-antigen interactions has made antibodies indispensable lab reagents.
- Antibodies are routinely used to detect proteins, identify cell populations, quantify soluble molecules, and investigate signalling pathways.
- Many of the most widely used techniques in modern molecular and cellular biology rely upon antibody-based detection systems.



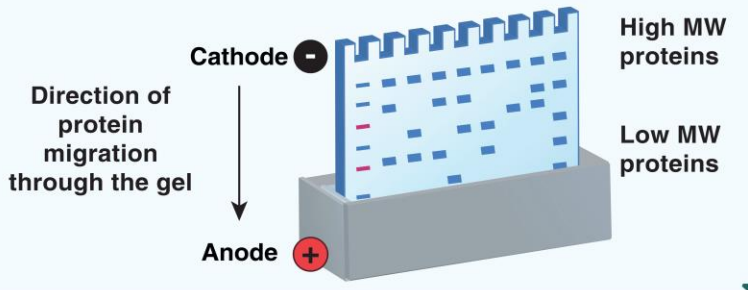
Western Blotting

- Western blotting allows specific proteins to be detected within complex biological samples.
- Proteins are first separated according to molecular weight using SDS-PAGE before being transferred to a membrane.
- Antibodies are then used to identify the protein of interest amongst thousands of other cellular proteins.
- The technique provides information about both protein expression and approximate molecular size.



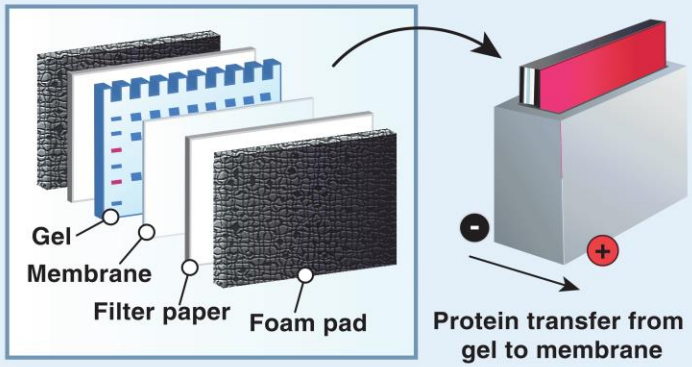
1 Sample preparation

Lyse the sample in an appropriate lysis buffer. Reduce and denature the sample with sample buffer containing SDS and β-mercaptoethanol. Heat the sample at 95°C 5 mins.



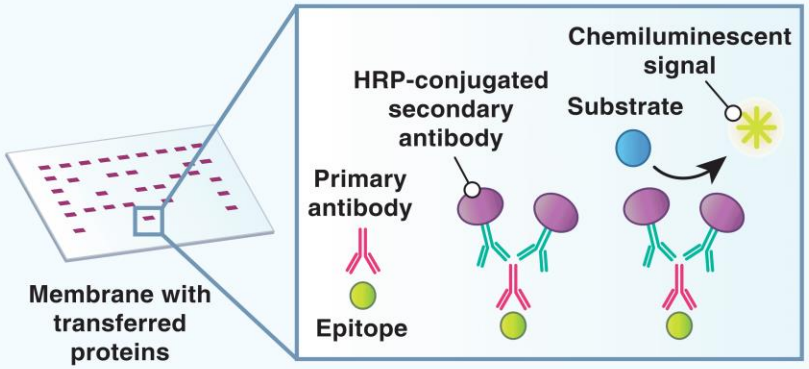
2 Gel electrophoresis

Load an optimized quantity of sample onto the gel. Perform gel electrophoresis at 100 - 200 V for 30 mins - 2 hrs. Proteins separate according to size, with smaller proteins migrating more quickly through the gel.



3 Membrane transfer

Prepare cold transfer buffer and the membrane. Assemble the transfer sandwich with the gel near the cathode (-) and the membrane near the anode (+). The negatively charged proteins will migrate out of the gel onto the membrane. Perform the transfer at 30 V O/N at 4°C or 100 V 1 hr.



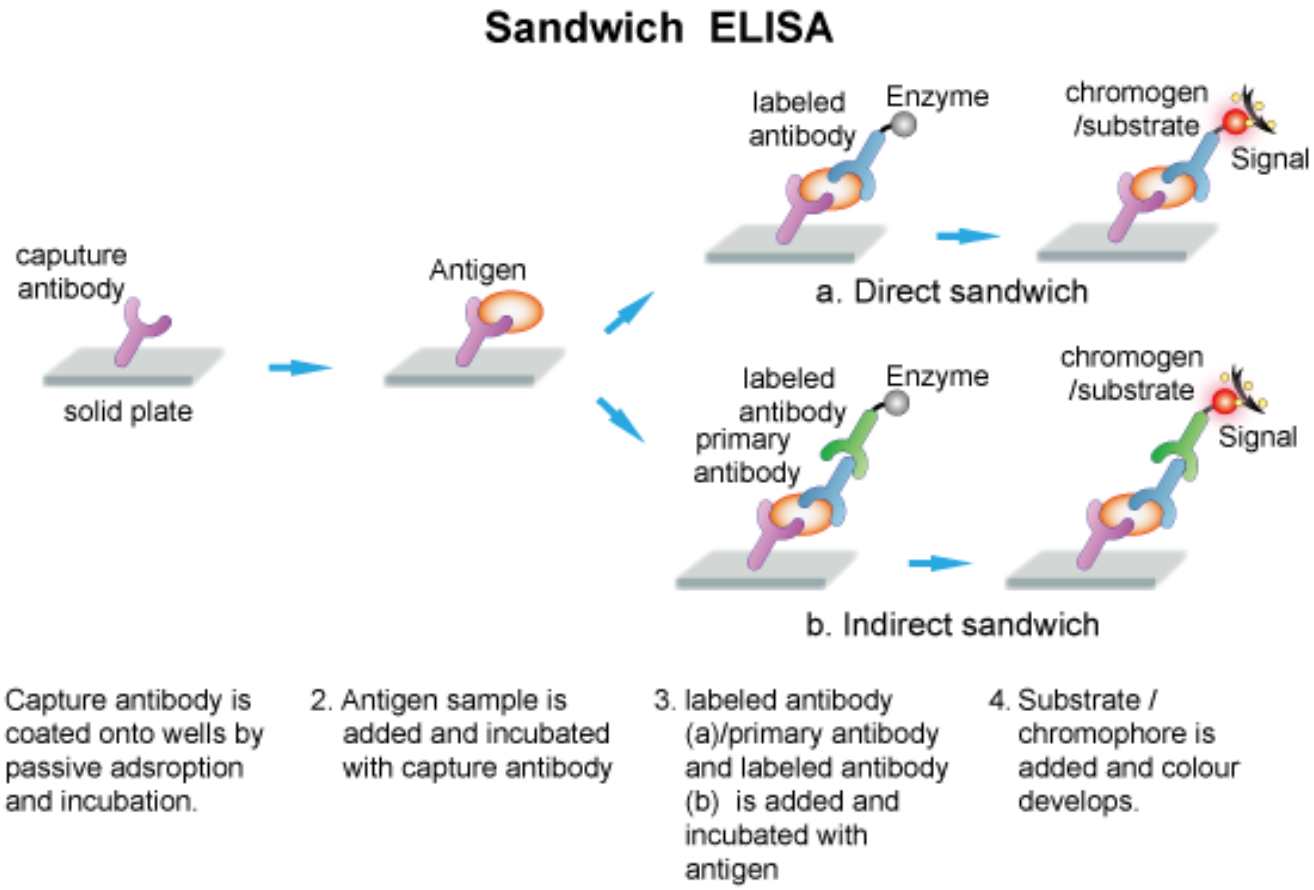
4 Immunodetection

Use a total protein stain to check the transfer efficiency. Block the membrane in an appropriate blocking buffer (milk, BSA, etc.). Incubate the membrane with primary antibody 1 hr RT or 4°C O/N. Incubate with secondary antibody 1 hr RT. Perform the detection step (ex. using a chemiluminescent substrate kit). Perform data analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

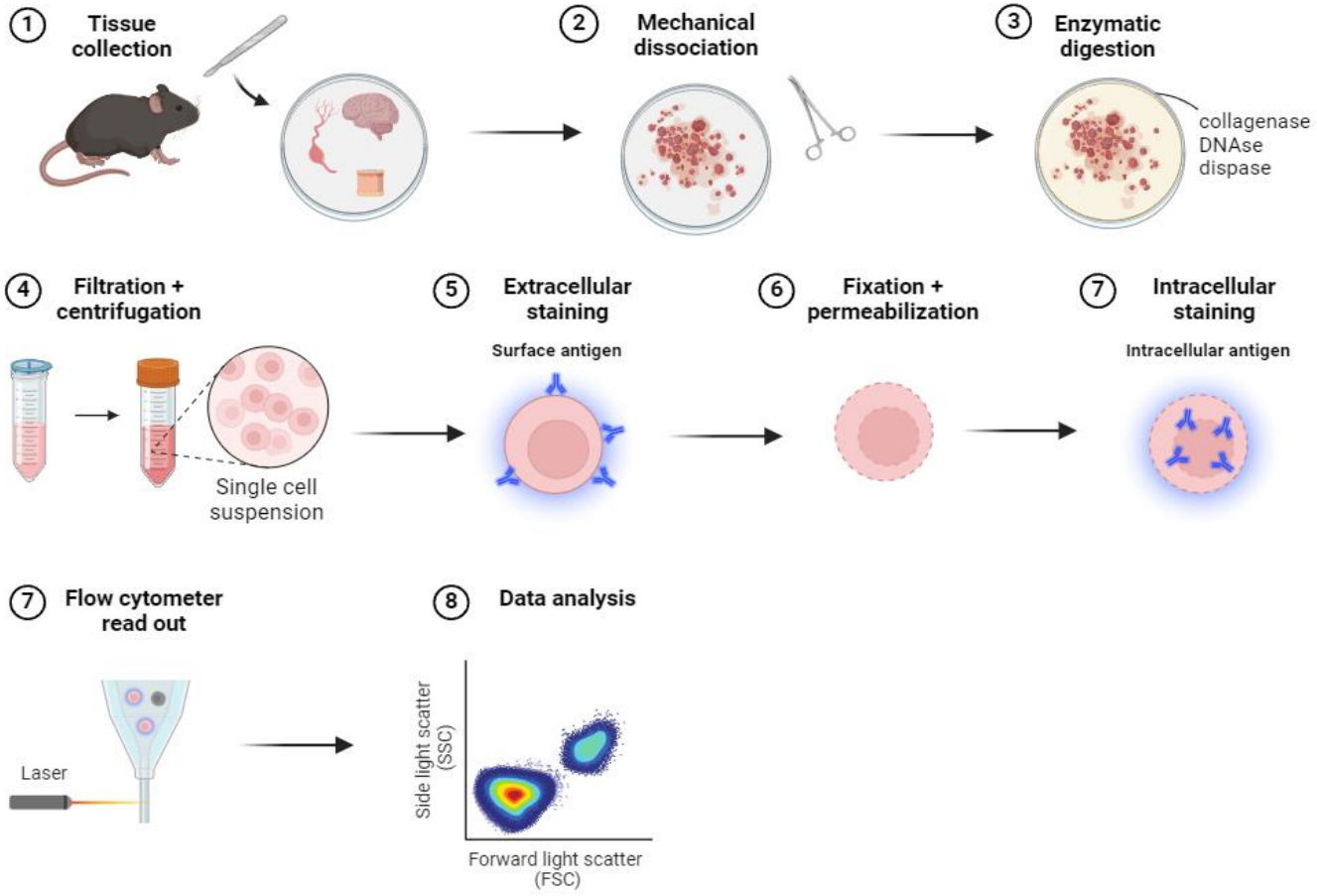
- ELISA is widely used for the detection and quantification of soluble molecules.
- In a sandwich ELISA, one antibody captures the antigen while a second antibody is used for detection.
- The amount of signal generated is proportional to the amount of antigen present within the sample.
- ELISA is commonly used to measure cytokines,

  hormones, biomarkers, and pathogen-derived proteins.



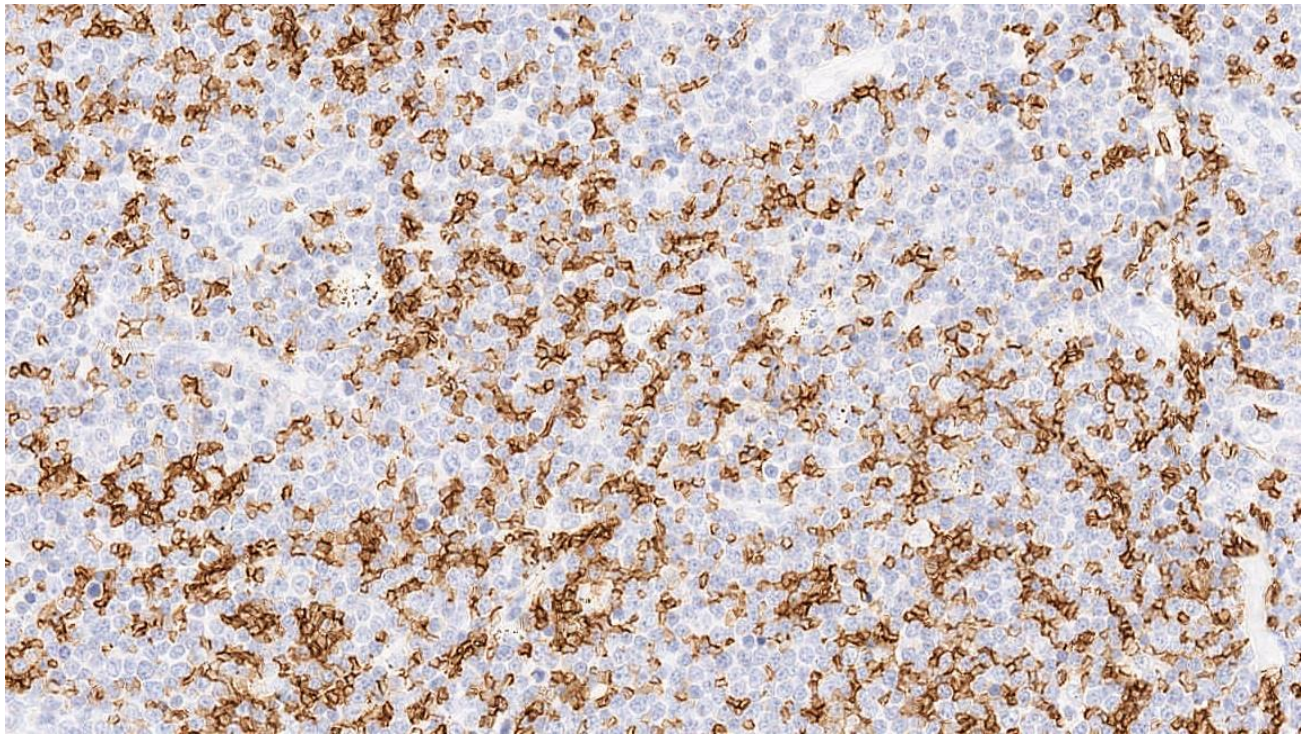
Flow Cytometry

- Different cell types express characteristic proteins on their surface.
- Fluorescently labelled antibodies can be used to detect these markers.
- Cells pass individually through a laser beam and the resulting fluorescence is measured.
- Multiple markers can be analysed simultaneously, allowing complex cell populations to be identified and quantified.



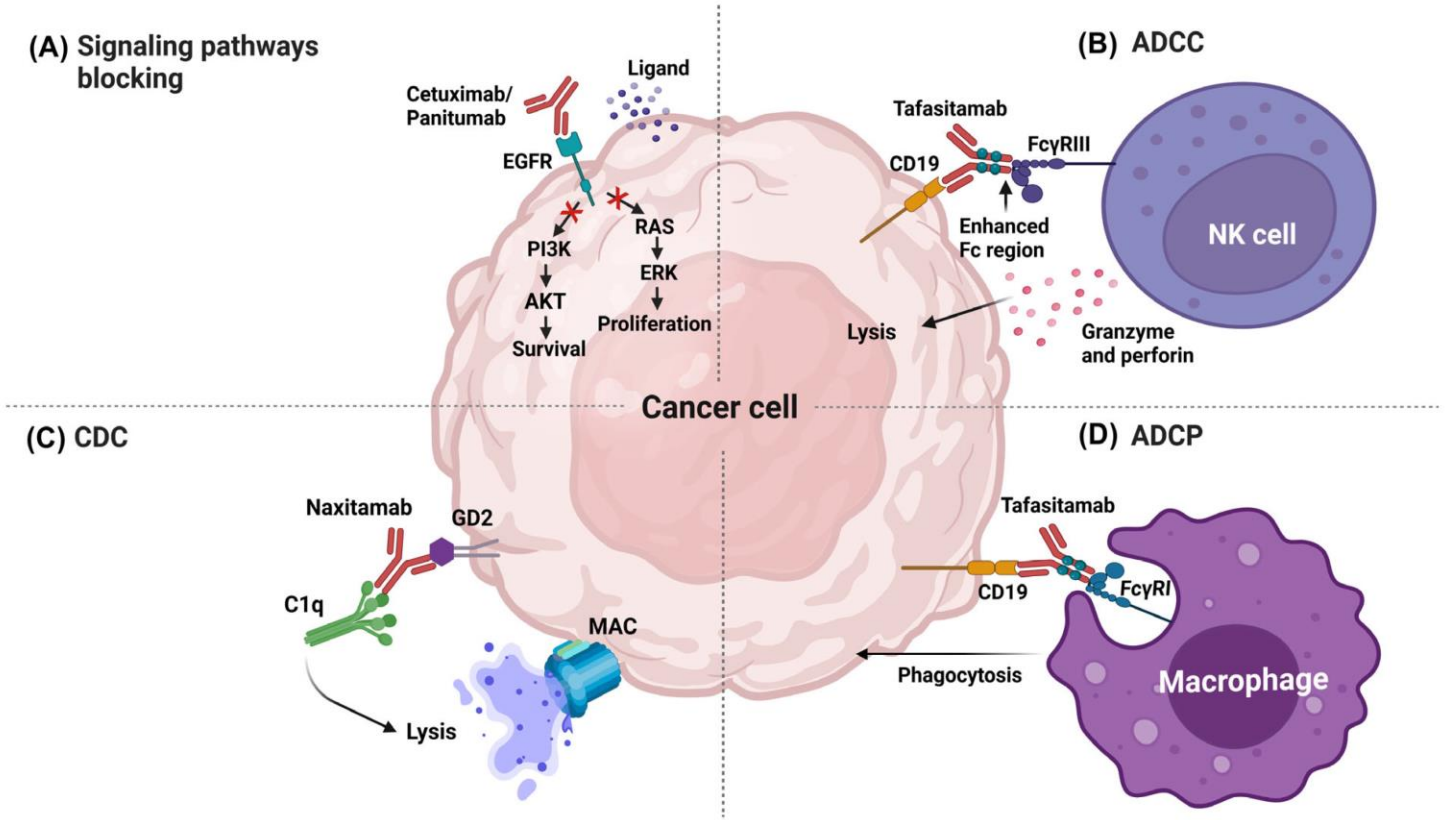
Immunohistochemistry

- Antibodies can be used to detect proteins directly within tissue sections.
- This allows researchers and pathologists to determine not only whether a protein is present, but also where it is expressed.
- Immunohistochemistry is widely used in cancer diagnostics, developmental biology, and pathology.
- The technique provides important spatial information that cannot be obtained from many other protein detection methods.



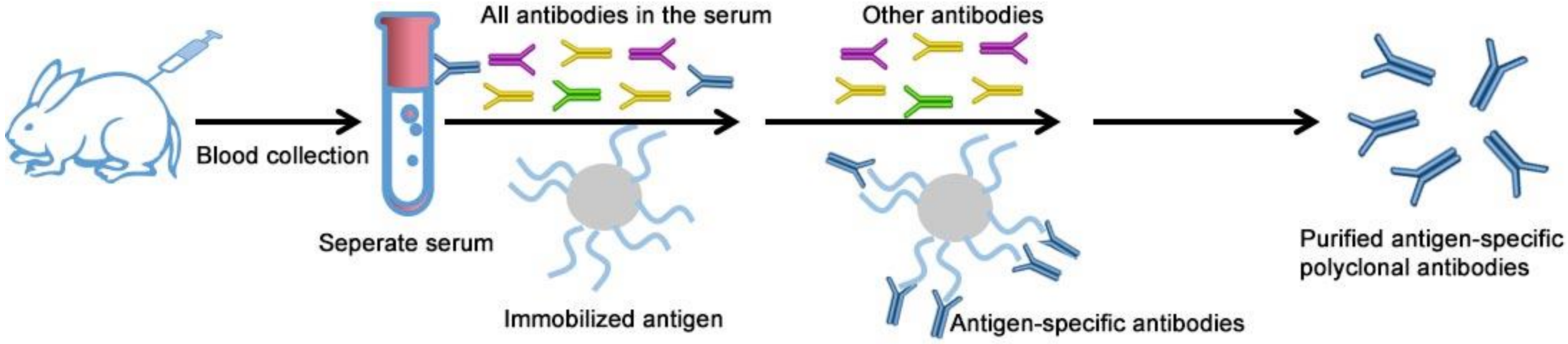
Therapeutic Applications of Antibodies

- Therapeutic antibodies can influence disease through several different mechanisms.
- Cetuximab and panitumumab bind EGFR and prevent receptor activation, thereby inhibiting downstream signalling pathways involved in tumour growth and proliferation.
- Tafasitamab targets CD19 expressed on malignant B-cells and promotes their elimination through antibody-dependent cellular cytotoxicity (ADCC) and other immune mechanisms.
- Naxitamab recognises GD2 on neuroblastoma cells and recruits immune effector cells to destroy the tumour.



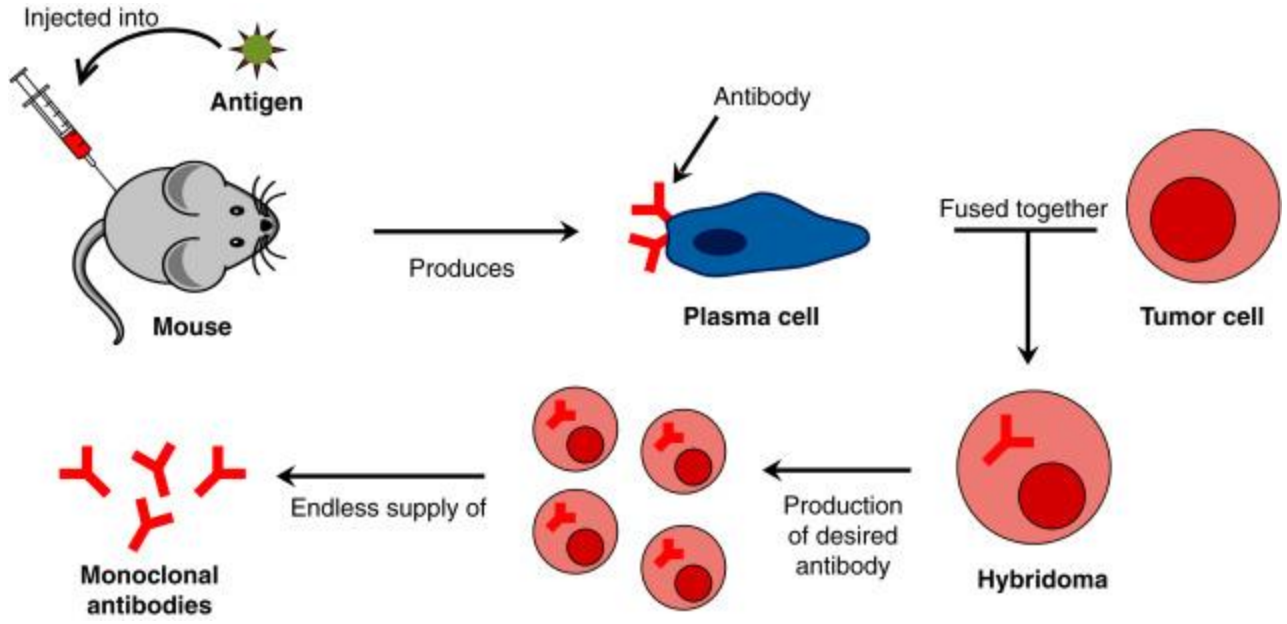
Polyclonal Antibodies

- Polyclonal antibodies are produced by multiple B-cell clones and therefore recognise several epitopes on the same antigen.
- Because multiple antibody populations contribute to binding, polyclonal preparations often generate strong overall signals and may remain effective when the target protein is partially degraded.
- Production typically involves immunisation of an animal followed by collection of antibody-containing serum.
- Since many different antibody molecules are present, batch-to-batch variation can occur and specificity may be lower than that achieved with monoclonal reagents.
- Despite these limitations, polyclonal antibodies remain widely used in research applications such as Western blotting and some ELISA assays.



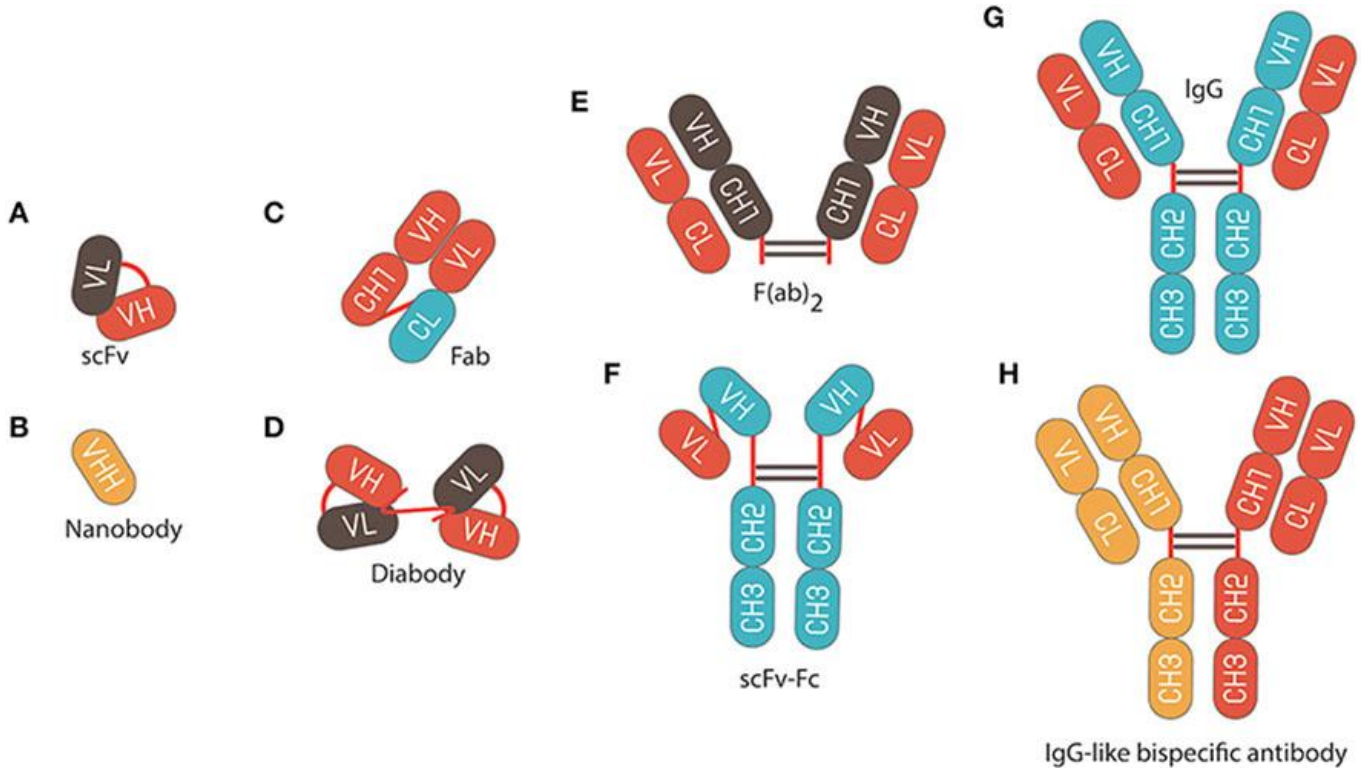
Monoclonal Antibodies

- Monoclonal antibodies originate from a single B-cell clone and therefore recognise a single epitope on the target antigen.
- Because all antibody molecules are identical, monoclonal reagents generally provide excellent specificity and reproducibility.
- Monoclonal antibodies are commonly generated using hybridoma technology, where antibody-producing B-cells are fused with immortal myeloma cells.
- Their consistency makes them particularly useful for flow cytometry, diagnostic testing, and therapeutic antibody development.
- Most clinically approved antibody drugs are monoclonal antibodies.



Antibody Fragments and Recombinant Antibodies

- Advances in molecular biology have enabled antibody genes to be cloned and expressed recombinantly.
- Smaller antibody-derived molecules such as Fab fragments and single-chain variable fragments (scFv) can retain antigen-binding activity while reducing molecular size.
- These formats are widely used in biotechnology, diagnostics, and cell-based therapies.
- Recombinant production also improves consistency compared with traditional antibody generation methods.



Summary

- Antibodies combine two highly desirable properties: exceptional specificity and exceptionally strong binding.
- Through genetic rearrangement and affinity maturation, the immune system can generate antibodies against an enormous range of molecular targets.
- These properties have made antibodies central to techniques such as Western blotting, ELISA, flow cytometry, and immunohistochemistry.
- The same principles have also enabled the development of antibody-based therapies for cancer, autoimmune disease, and chronic inflammatory disorders.
- Advances in recombinant antibody engineering continue to expand the role of antibodies in both research and medicine.

Quiz

- Why can the immune system generate millions of antibody specificities without requiring millions of genes?
- Why are monoclonal antibodies generally preferred for therapeutic applications?
- Why might an antibody perform well in ELISA but poorly in Western blotting?
- How does affinity maturation improve the quality of an antibody response?
- What advantages do recombinant antibody fragments offer over conventional IgG molecules?