

Assignment of Cellular Perspective

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## Context:

* Experimental Methodologies in Cell Biology
* Visualization of Cell by Microscopy
* Light Microscope /Electron Microscope/FACS
* Polyclonal and Monoclonal Antibodies

Experimental Methodology

# Experimental Methodologies in cell biology:

Science proceeds by use of the experimental method .This general method is used not only in biology but in chemistry, physics, geology and other hard science. To gather the information about the biological world, we use two mechanisms: our sensory perception and our ability to reason.

Some methodologies used in cell biology are as follow:

* Microscopy visualization
* Spectrophotometry
* Fluorescence
* Radiochemistry
* Differential precipitation of proteins
* Chromatography
* Electrophoresis
* Immunoassay
* Hybridization
* Blotting Techniques

# Visualization of Cell by Microscopy:

### Introduction to visualization of cell:

Cell is the basic functional and structural unit of life. Human eye cannot see a normal cell by naked eye. In order to study and understand all the process taking place inside a cell the study of cell structure is mandatory. In this regard great efforts were done by scientists one of which is light microscope or optical microscope.

To study the micro sized cell and its organelles different process has been used including;

Optical microscope, electron microscope, fluorescence activated cell sorting (FACS), green fluorescence protein (GFP)

### Size:

Typical cell is of 20 micrometre

Large organelle 2 micrometre

Macromolecule complexes 0.2 micrometre

### Microscopy:

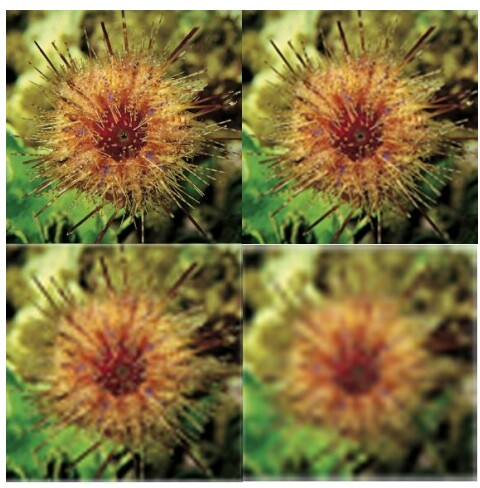
‘The use of a microscope to visualize the given sample or specimen is called microscopy’’

I.e. Light Microscopy, Electron Microscopy, Fluorescence Microscopy

### Resolution and Magnification

### Resolution:

‘’The ability of microscope to distinguish between **two very small** and **closely placed** points’’



#### Resolution of a lens

* Resolution is best when the distance between two separating tiny object is small
* It is the degree to which detail in specimen is retained in magnified image
* Resolving power; unaided eye **0.1 mm** apart, microscope **0.2 micrometre** apart

### Magnification:

‘The ability of a microscope to zoom in and increase in size of the obtained image of specimen’’

The magnification depends upon the lens.

# Light Microscope

### Introduction:

The light microscope or optical microscope is a type of microscope that commonly uses **visible light** ranging wavelength from **360-780nm.**A system of lenses is used to generate image of a small object.

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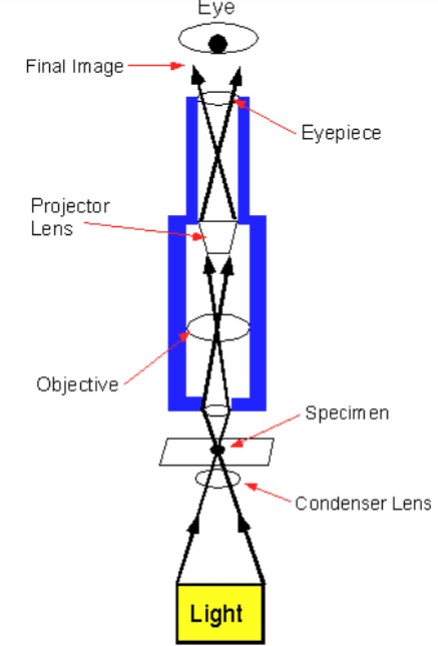
##### A light microscope

### Principle of light microscope

The light microscope is an instrument for visualization of detail of an object .It does this by creating a magnified image through the use of series of glass lenses, which first focus a **beam of light** onto or through an object, and convex objective lens to enlarge the image formed.

### Working

A light microscope uses visible light diffracted through the mirror. The light passes through the specimen and magnifies its image. There used two lenses in a light microscope **objective lens** and **eyepiece.**

* **Objective lens** is near the specimen and first magnified image is formed
* **Eyepiece** is near the eye where is to observe the enlarged image of the sample
* **Slide** is used to place the specimen on and observe under the light microscope****

##### Working of light microscope

* **Magnification** of light microscope is **1500X**
* **Resolving power** of light microscope is **0.2micrometre**

### Applications of Light Microscope

* Light microscope play a large role in today’s biology
* Biologists use light microscope to observe objects and details at a cellular level to learn more about the building blocks of all organisms
* Microscopes are also used to observe real time movement in cell and organisms

### Advantages

* Inexpensive to buy and operate
* Relatively small
* Both living and dead specimen can be viewed
* Little expertise is required in order to set up and use the microscope
* The original colour of the specimen can be viewed

### Limitations

* Maximum magnification of **1500X**
* Specimen may be disfigured during the preparation to be viewed under the microscope
* The resolving power is **1nm** for biological specimen
* Only has a resolution of **0.2micrometre** which is relatively poor than other microscopes

# Electron Microscope

### Introduction

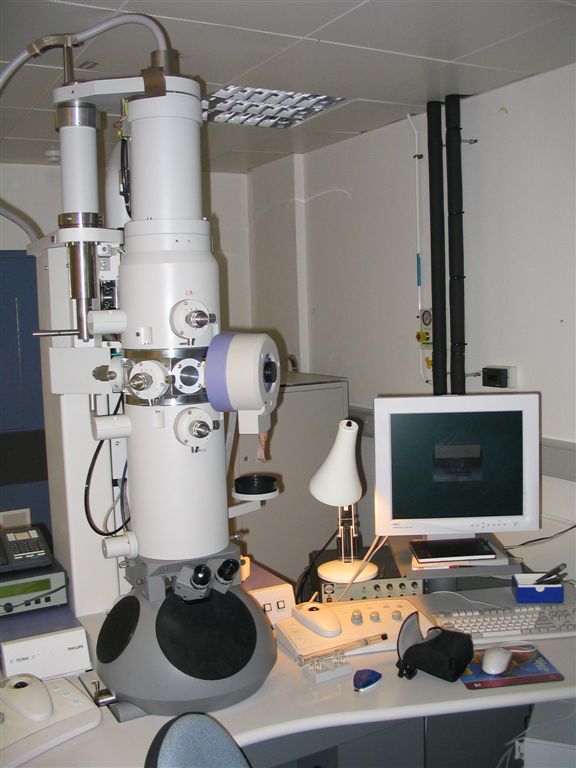
An electron microscope is a high magnification and resolution microscope that uses the **electron beam** to visualize the specimen. It uses electrons to fall on the specimen and to create a highly magnified picture of the specimen. The organelles and the processes taking place in the organelles are studied under electron microscope.

### Definition

‘An electron microscope is a microscope that uses the **beam of electrons** to visualize the cell organelle structure and the molecular level of specimen’’

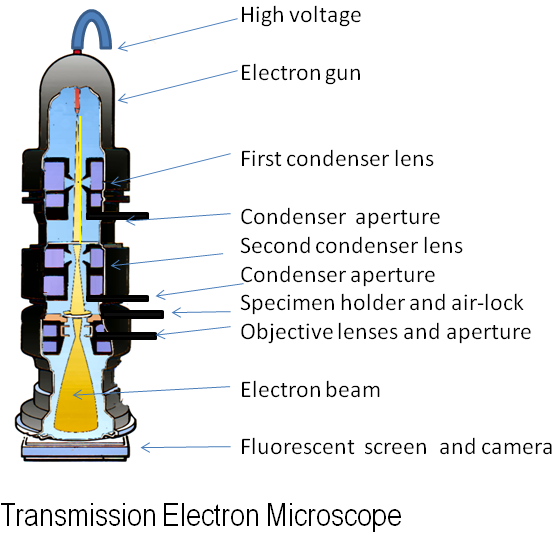
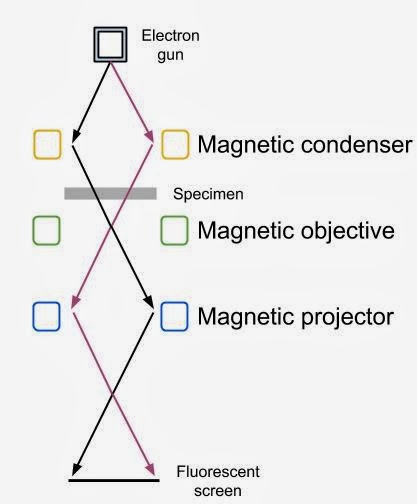
### Principle

Accelerated electrons from SEM carry significant amount of kinetic energy and this energy is replaced by as a variety of signals to obtain information about structure, morphology and composition.

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### Working

* The electron gun generates electron beam
* Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam
* To move electron down the column, an accelerating voltage (mostly between **100Kv-1000kV**) is applied between tungsten filament and anode.
* The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope
* Ultra-thin section of 20-100nm are cut which is already placed on the specimen holder.
* The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen
* The electron beam coming out of the specimen passes to objective lens ,which has high power and forms the intermediate magnified image
* The ocular lenses then produce the final further magnified image



### Applications

* Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimen including microorganisms, cells, large molecules, biopsy samples, metals and crystals.
* Industrially, electron microscopes are often used for quality control and failure analysis.
* Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the images.
* Science of microbiology owes its development to the electron microscope. Study of microorganisms like bacteria, virus and other pathogens has made the treatment of diseases very effective.

### Advantages

* Very high magnification
* Incredible high resolution
* Material rarely distorted by preparation
* It is possible to investigate a great depth of field
* Diverse applications

### Limitations

* The live specimen cannot be observed
* As the penetration power of the electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.
* As the EM works in a vacuum , the specimen should be completely dry
* Expensive to build and maintain
* Requiring researcher training
* Image artefacts resulting from specimen preparation
* This type of microscope is a large, cumbersome extremely sensitive to vibration and external magnetic field.

# Fluorescence Activated Cell Sorting (FACS)

### Introduction

The flow cytometer or fluorescence activated cell sorting (FACS) is a popular cell biology technique that utilizes laser-based technology to count, sort and profile cell in a heterogeneous fluid mixture.

### Definition

‘Fluorescence activated sorting (FACS) is a special type of flow cytometer and is used for the evaluation of peptides and DNA in addition to membranes and intercellular protein.’’

### Principle

By utilizing highly specific antibodies labelled with fluorescent conjugates, FACS analysis allows simultaneously collect data on and sorts a biological sample by a nearly limitless number of different parameters.

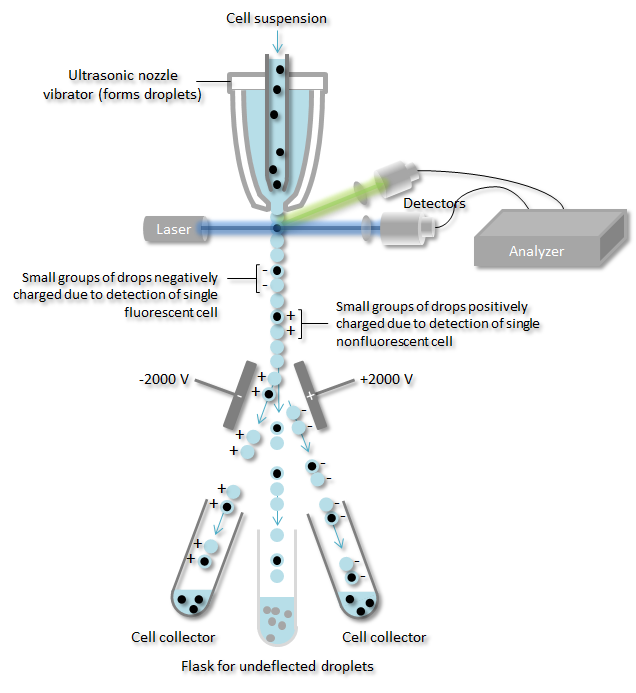
Cells are dyed with a fluorescent antibody, and then placed in a stream of liquid which passes the focus of a laser and each cell emits light.

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#### Fluorescent activated cell sorting principle

## Working

* Individual cells are **interrogated** by the laser as in **a** normal flow cytometer.
* The machine is set up so that each individual cell then enters a single droplet as it leaves the **nozzle** tip. This drop is given an electronic charge, depending on the fluorescence of the cell inside the drop.
* Deflection plates attract or repel the cells accordingly into collection tubes.
* **A single FITC stained cell** in a single droplet would be given a positive charge and be attracted to the right. Collection tube to the right would collect all the positively charged FITC stained cell droplets.
* **A single PE stained cell** in a single droplet would be given a negative charge and be attractive to the left. Collection tube to the left would collect all the PE stained cell droplets.
* **Sorted cell population** then analysed to ensure successful cell sorting.
* Sorted cells then are cultured.



#### Fluorescence activated cell sorting working

### Applications

* FACS is an essential tool for efficient screening for novel microalga strains.
* Microalga strain improvement can be performed by FACS rounds.
* FACS can be used to remove contaminants from valuable microalga cultures.
* Recent trends indicate that FACS can also be used for metabolic monitoring.
* FACS is preferred method when very high purity of desired population is required, when the target cell population express a very low level of the identifying marker or when cell populations require separation based on differential marker density.
* FACS is the only available purification technique to isolate cells based on internal staining or intercellular protein expression, such as a genetically modified fluorescent protein marker.
* FACS allows the purification of individual cell based on size, granularity and fluorescence. In order to purify cell of interest, they are

### Advantages

* Fast
* More than two types of cell can be isolated simultaneously
* High purity
* Isolation can be performed based on either surface or intracellular protein s high throughput
* Cell with different levels of marker expression can be isolated
* Separation of rare cell populations

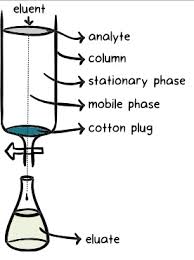
### Limitations

* Expensive
* Skilled staff is needed
* Preparing a single suspense is a critical prerequisite

# Cell Chromatography

### Introduction

Cell chromatography is used to separate and purify many kinds of compounds, including carbohydrates, lipids and nucleic acids. For biological molecules, **liquid chromatography** is the technology used most often; the most common target molecule separated by liquid chromatography is a **particular protein.** Normally chromatography will separate the molecules from other molecule in a mixture based on a few characteristics such as **affinity, size and charge.**

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### Types of chromatography

Types of chromatography used in cell biology are as follows:

* Ion-exchange chromatography
* Affinity chromatography
* Partition and adsorption chromatography
* Gel filtration chromatography

##### Ion-exchange method

In this method, molecules are separated on the bases of difference in charge. Many biological macromolecules such as **amino acids and proteins** have ionisable groups. They may carry positive or negative charge .The charge showed by these groups depend on the pH of the solution.

##### Affinity chromatography

In this method, the property of biological interactions between the molecules is used in order to get separation and purification. In this case some ligand molecules i.e. subtract of an enzyme or some antibody is bound to the matrix of the column.

##### Partition and adsorption chromatography

It is a common practice to separate many substances by shaking the substance in two immiscible liquid phases in a separating funnel. When a substance is shaken in a solvent it will partition with the two phases. If one phase is allowed to move the substance will also move on the basis of its partition coefficient.

##### Gel-filtration chromatography

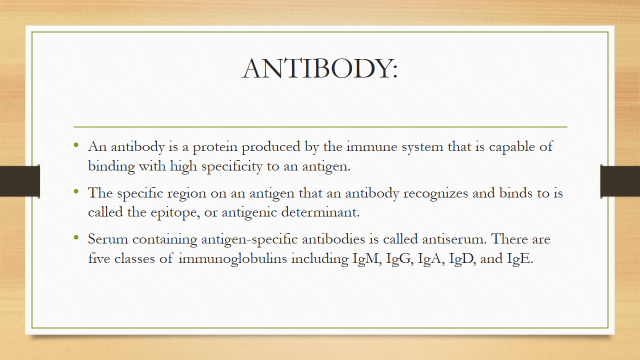
This type of chromatic separation takes place on the basis of the **size and shape** of molecule utilizing the porosity of the **gel material.** This method is also known as **exclusion or permeation chromatography.**

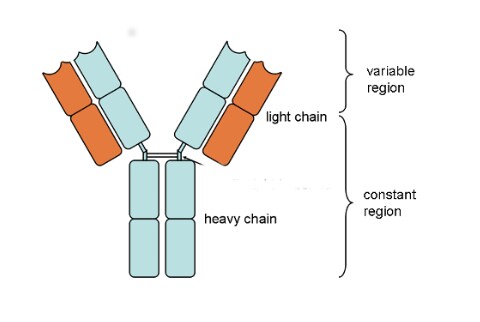
Polyclonal and monoclonal antibodies

### Introduction to antibody

Antibodies, also known as **immunoglobulin,** are secreted by B cell to neutralize antigens such as bacteria and virus.

The classical representation of an antibody is **Y shaped** molecule composed of **four polypeptide (two heavy and two light) chains** Each tip of the **Y** contains a **paratrooper** ( a structure analogous to a lock) that is specific for one particular **epitope** (structure analogous to a key) on an antigen, allowing these two structures to bind together with precision.





### Types of antibodies

These antibodies can be classified into two primary types

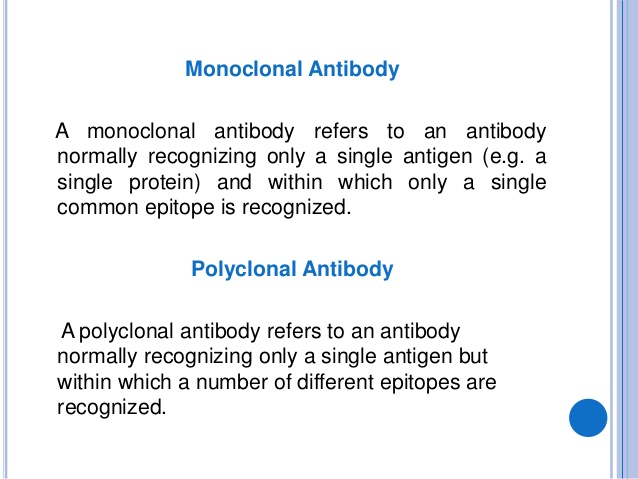
* **Monoclonal antibodies**
* **Polyclonal antibodies**

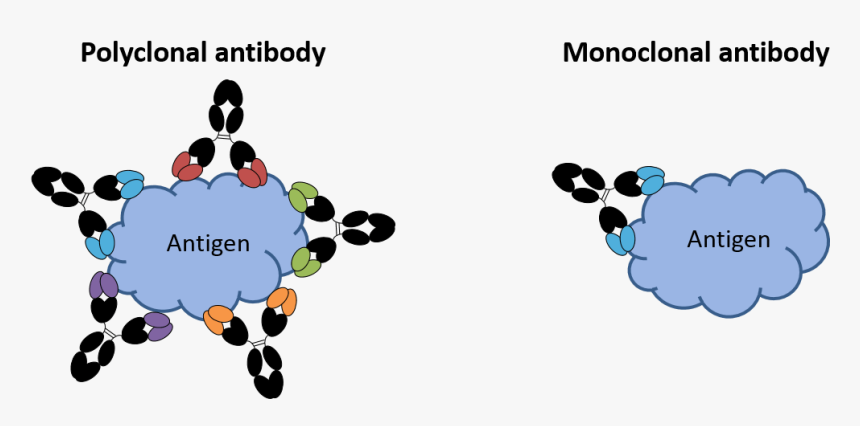
These are classified by the means in which they are created from lymphocytes. Each of them has important role in immune system, diagnostic exams, and treatments.

**What is a Polyclonal Antibody?**  
A Polyclonal Antibody represents a collection of antibodies from different B cells that recognize multiple epitopes on the same antigen. Each of these individual antibodies recognizes a unique epitope that is located on that [antigen](http://www.pacificimmunology.com/resources/antigens/).

**What is a Monoclonal Antibody?**  
A Monoclonal antibody, by contrast, represents antibody from a single antibody producing B cell and therefore only binds with one unique epitope. Each individual antibody in a polyclonal mixture is technically a monoclonal antibody; however, this term generally refers to a process by which the actual B-cell is isolated and fused to an immortal hybridoma cell line so that large quantities of identical antibody can be generated.

**OR**

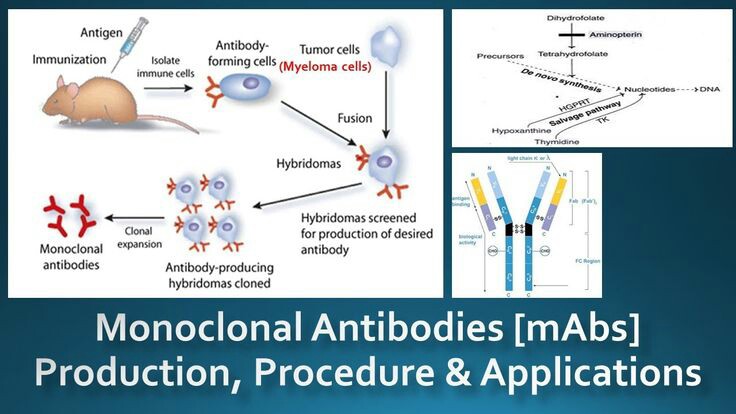




## Production of antibodies (Not included)

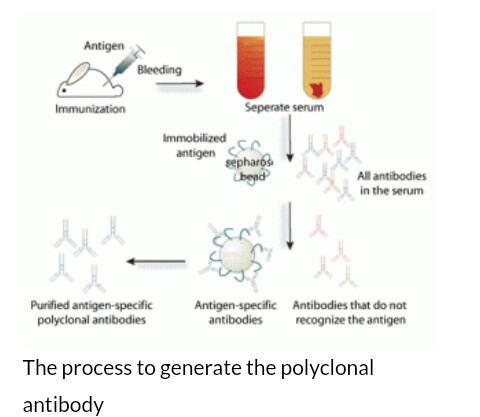
### Production of monoclonal antibodies

* Monoclonal antibodies (abs) are generated by **identical B cells** which are clones from a single parent cell.
* The monoclonal antibodies have **monovalent affinity** and only recognize the same epitope of an antigen.
* Monoclonal antibodies are produced ***ex-vivo*** using tissue culture techniques.
* The process begins with an injection of desired antigen into an animal, often a mouse, multiple times.
* Once the animal **develops** an **immune response**, the B lymphocytes are isolated from the animal’s spleen and fused with a **myeloma cell line**, creating immortalized B cell myeloma hybridises.
* The **hybridises (**which are able to grow continuously in culture while producing antibodies) are then screened for desired mAbs.



### Production of polyclonal antibodies(NOT INCULDED)

* Polyclonal antibodies (pAbs) are mixture of **heterogeneous** which are usually produced by **different B-cell clones** in the body.
* They are recognized and bind to **many different epitope of a single antigen.**
* Polyclonal antibodies are produced by **injecting an immunogen** into an animal.
* After being injecting with a **specific antigen** to elicit a **primary immune response**
* After immunization, polyclonal antibodies can be obtained straight from the serum proteins.



## Advantages and disadvantages of monoclonal antibodies

### Advantages:

* Batch-to-batch reproducibility (**high homogeneity**)
* Possibility to produce large quantities of **identical antibody** ( an advantage for diagnostic manufacturing and therapeutic drug development)
* **High specificity** to a single epitope reflected in low cross-reactivity.
* **More sensitive** in assays requiring quantification of protein levels.

### Disadvantages:

* More **expensive** to produce. It is necessary to produce a pool of several monoclonal antibodies.
* Require significantly **more time** to produce and develop the hybridized clone(+/- 6 months)
* More **susceptible to binding changes** when labelled.

## Advantages and disadvantages of polyclonal antibodies

### Advantages:

* **Inexpensive** and relatively **quick** to produce(+/- 3 months)
* **Higher overall antibody** affinity against the antigen due to recognition of multiple epitopes.
* Have a **high sensitivity** for detecting low quantity proteins.
* High ability to capture the target protein
* Antibody affinity results in **quicker binding** to the target antigen.
* Superior for use in **detecting a native protein**.
* **Easy to couple with antibody** labels and rather unlikely to affect binding capability.

### Disadvantages:

* **Batch-to-batch variability** as produced in different animals at different times.
* **High chance of cross-reactivity** due to recognition of multiple epitopes.

