

# Edexcel A Biology A-Level

## Core Practical 5

Prepare and stain a root tip squash to observe the stages of mitosis.



Plant cells undergo mitosis at shoot and root tips in areas called **meristems**. Cells in the meristems are **totipotent** and retain the ability to **differentiate**.

The **mitotic index** of a sample is the **ratio** of cells **undergoing mitosis** to the **total number of cells** in a sample. To find the **mitotic index**, cells from the meristem must be viewed under an **optical microscope**.

## Equipment

- Garlic root tip
- 1M hydrochloric acid
- Toluidine blue stain
- Distilled water
- Watch glasses
- Sample tube
- Pipettes
- Microscope slides and coverslips
- Forceps
- Filter paper
- Scissors
- Optical microscope

## Method

1. Cut a 5mm sample of the **root tip** using a **scalpel**.
2. Transfer root tip to sample tubes containing **HCl** and leave for 5 minutes.
3. Transfer to watch glass containing cold distilled water. Leave for 5 minutes.
4. Dry root tips on filter paper.
5. Place tip on a microscope slide. **Macerate** with a needle to spread the cells out. This makes the chromosomes visible and will therefore show which cells are undergoing mitosis.
6. Add a drop of **toluidine blue** to the slide and leave to stain for **2 minutes**.
7. Lower the cover slip down carefully onto the slide. Make sure there are **no air bubbles** in the slide which may distort the image, and that the coverslip doesn't slide sideways which could damage the chromosomes.
8. Wrap in a paper towel and gently 'squash' the slide.
9. Place under a microscope and set the **objective lens** on the lowest magnification, then use the **coarse adjustment knob** to move the lens down to just above the slide.
10. Use the **fine adjustment knob** to carefully re-adjust the focus until the image is **clear** (can use a higher magnification if needed).





11. To calculate **mitotic index**, cells **undergoing mitosis** must be counted (cells with **chromosomes visible**), as well as the **total number of cells**.

$$\text{Mitotic index} = \frac{\text{number of cells with visible chromosomes}}{\text{total number of cells in sample}}$$

## Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Hydrochloric acid	May cause harm/irritation to eyes or in cuts	Wear eye protection; avoid contact with skin, keep away from edge of desk	Wash off skin immediately; flood eye/cuts with cold water	Low
Toluidine blue O stain	May cause harm/irritation to eyes or in cuts	Wear eye protection; avoid contact with skin	Wash off skin immediately; flood eye/cuts with cold water	Low
Scalpel	Cuts from sharp object	Cut away from fingers; use forceps to hold sample whilst cutting, keep away from edge of desk	Elevate cuts; apply pressure; seek medical assistance	Low
Broken glass	Cuts from sharp object	Take care when handling slides and coverslips; keep glassware away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low

