

AQA Biology A-Level

Required Practical 2

Preparation of stained squashes of cells from plant root tips;
set-up and use of an optical microscope to identify the
stages of mitosis in these stained squashes and calculation
of a mitotic index.



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 list

- Optical microscope
- Microscope slides and cover slips
- Water bath
- Hydrochloric acid
- Toluidine blue O stain
- Distilled water
- Scalpel
- Forceps
- 100 ml beaker
- Root tip

Method

1. Heat 1 mol dm⁻³ HCl at **60°C** in a water bath.
2. Cut a small sample of the **root tip** using a **scalpel**.
3. Transfer root tip to **HCl** and **incubate** for 5 minutes.
4. Remove from HCl and wash sample in cold distilled water and **remove the very tip** using a scalpel.
5. Place tip on a microscope slide and add a few drops of **stain** (e.g. **toluidine blue O**). This makes the **chromosomes visible** and will therefore show which cells are undergoing mitosis.
6. 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.
7. Place under a microscope and set the **objective lens** on the lowest magnification.
8. Use the **coarse adjustment knob** to move the lens down to just above the slide.



9. Use the **fine adjustment knob** to carefully re-adjust the focus until the image is **clear** (you can use a higher magnification if needed).
10. 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, tie up long hair	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 the edge of the 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

