
A-level Biology example for 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:

Root tip squash using onion root meristem tissue

Student sheet

You are provided with the following:

- 100 ml beaker
- hydrochloric acid (5 mol dm^{-3})
- microscope slide and cover slip
- toluidene blue stain
- filter paper
- mounted needle
- scalpel
- distilled water
- watch glass
- forceps
- root tip of onion or garlic
- piece of white paper
- paper towel
- microscope and light source.

You are required to prepare a microscope slide of the meristem tissue from an onion root. You will add a stain to the material which allows you to see the chromosomes. You will look at the slide under the microscope to identify any cells showing stages of mitosis. You will then calculate the mitotic index.

Safety

Hydrochloric acid (5 mol dm^{-3}) is corrosive and you should handle it with caution. You must wear eye protection.

You must stand the beaker on a bench mat. Do not carry the beaker with acid in it.

Do not leave root tips for investigation lying on the bench top prior to staining. Cut your root tip immediately before you put it into the acid. This will stop any reactions and hopefully some cells will be in a stage of division.

You should read these instructions carefully before you start work.

Making your slide

1. Stand the beaker on a bench mat before adding approximately 10 ml of hydrochloric acid (5 mol dm^{-3}). Put some paper towel on the bench mat and label.
2. Place about 2cm of root tip in the acid and leave for 15 minutes.
3. Set up your microscope while you are waiting.
4. Rinse the root tip in distilled water in the watch glass.
5. Cut off the root tip (1mm) and place on a microscope slide.
6. Cover the section with toluidene blue stain and macerate with the mounted needle to separate the cells. Use a piece of white paper to aid colouration of roots.
7. Continue to macerate until the tissue is well broken and the cells are stained dark blue.
8. Add a cover slip and with gentle finger pressure 'spread' the material and blot at the same time by using a folded filter paper between finger and slide.
9. Look carefully at all slides for cells undergoing mitosis. Chromosomes should stain dark blue. Repeat for several fields of view.
10. Record your data in a suitable table.
11. Calculate the mitotic index.