A-level Biology example for required practical 8

Investigation into the effect of a named factor on the rate of dehydrogenase activity in extracts of chloroplasts: The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP

Student sheet

In this investigation you will use a chloroplast suspension and a blue dye called DCPIP to monitor the rate of dehydrogenase activity. DCPIP goes from blue to colourless when it takes up electrons released by chlorophyll.

Method

You are provided with the following:

- Spinach leaves
- access to a blender
- measuring cylinder
- muslin (or material for filtering)
- filter funnel
- three beakers
- ice
- isolation medium (cold)
- DCPIP solution (cold)
- distilled water (cold)
- ammonium hydroxide solution (cold)
- test tubes
- test-tube rack
- syringes ($1cm^3$ and $5cm^3$)
- piece of aluminium foil
- lamp
- marker pen
- timer.

You should read these instructions carefully before you start work.

1. Put about $50cm^3$ of isolation medium into a beaker.
2. Tear eight spinach leaves into small pieces and put the pieces into the isolation medium in the beaker. Do not put pieces of the midrib or the leaf stalk into the beaker.
3. Half-fill a large beaker with ice and place a small beaker on top of the ice.
4. Put three layers of muslin over the top of the filter funnel and wet it with the isolation medium. Rest the filter funnel in the small beaker on the ice.
5. Pour the spinach and isolation medium into the blender and blend for about 15 seconds. Pour the blended mixture back into the beaker.
6. Pour a little of your blended mixture through the muslin in the filter funnel. Carefully fold and squeeze the muslin to assist the filtering process. Repeat until most of the blended mixture has been filtered. Label this filtrate in the small beaker on ice as 'chloroplast suspension'.
7. Label five test tubes **A**, **B**, **C**, **X** and **Y**. Stand these five tubes in the ice in the large beaker. Position the lamp about 10cm from the beaker so that all tubes are illuminated. Turn on the lamp.
8. Set up tubes **A** and **B** as follows:
   Tube A
   Put $5cm^3$ DCPIP solution + $1cm^3$ water + $1cm^3$ chloroplast suspension in the tube. Immediately wrap the tube completely in aluminium foil to exclude light.
   Tube B
   Put $5cm^3$ DCPIP solution + $1cm^3$ water + $1cm^3$ isolation medium in the tube.

Tubes **A** and **B** are control experiments. Leave both tubes until the end of your investigation.

9. Set up tube **C** as follows:

   Tube C

   Put 6cm^3 water + 1cm^3 chloroplast suspension in the tube.

   Tube **C** is for you to use as a standard to help you to determine when any colour change is complete.

10. Set up tube **X** as follows:

    Tube X

    Put 5cm^3 DCPIP solution + 1cm^3 water in the tube.

    Add 1cm^3 chloroplast suspension to tube **X**, quickly mix the contents and start the timer.

    Record in seconds how long it takes for the contents of tube **X** to change colour from blue-green to green. This is when all signs of blue have disappeared. Use tube **C** to help you determine when the colour change is complete.

11. Repeat step 10 four more times.

12. Set up tube **Y** as follows:

    Tube Y

    Put 5cm^3 DCPIP solution + 1cm^3 ammonium hydroxide in the tube.

    Add 1cm^3 chloroplast suspension to tube **Y**, quickly mix the contents and start the timer.

    Record in seconds how long it takes for the contents of tube **Y** to change colour from blue-green to green. This is when all signs of blue have disappeared. Use tube **C** to help you determine when the colour change is complete. However if this has not taken place within 300 seconds (five minutes), record the colour at this point.

13. Repeat step 12 four more times.

14. Record your data in a suitable table.

15. At the end of your investigation, record the colour of the mixtures in tubes **A** and **B**.