



AQA Biology A-Level Required Practical 8

Investigation into the effect of a named factor on the rate of dehydrogenase activity in extracts of chloroplasts.





Dehydrogenase is an **enzyme** found in plant chloroplasts that is crucial to the **light dependent stage** of photosynthesis. In the light dependent stage, **electrons** are accepted by **NADP**. Dehydrogenase **cataylises** this reaction.

When a **redox indicator dye** is present, such as **DCPIP**, electrons are accepted by this instead. The activity of dehydrogenase can therefore be investigated using DCPIP, which turns from **blue to colourless** when it is reduced.

Equipment list

- Leaf sample
- Isolation solution
- Ice water bath
- Distilled water
- DCPIP
- Pestle and mortar
- Test tubes
- Test tube rack
- Syringes
- Pipettes
- Lamp
- Timer
- Tape measure
- Muslin cloth
- Funnel
- Beaker
- Centrifuge
- Centrifuge tubes
- Colorimeter
- Cuvettes

Method

In this method the named variable is light intensity

1. **Remove stalks** from leaf samples. Grind sample using a pestle and mortar and place into a **chilled isolation solution**.
2. Use a muslin cloth and funnel to filter the sample into a beaker. Suspend the beaker in an **ice water bath** to keep sample chilled.



3. Transfer to centrifuge tubes and **centrifuge at high speed for 10 minutes**. This will **separate chloroplasts** into the **pellet**.
4. Remove **supernatant** and add pellet to the fresh isolation medium. Store isolation solution on ice.
5. Set the colorimeter to the **red filter**. **Zero** using a cuvette containing **chloroplast extract** and **distilled water**.
6. Place test tube in the rack 30cm from light source and add **DCPIP**. Immediately take a sample and add to cuvette. Measure the **absorbance** of the sample using the colorimeter.
7. Take a sample and measure its absorbance **every 2 minutes for 10 minutes**.
8. Repeat for different distances from lamp up to 100 cm. This will vary the **light intensity**.

NB: This experiment should be done in a **darkened room** to make results more reliable. The sample should not be put too close to the lamp as **temperature** may affect results.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
DCPIP	Irritant to skin and eyes; may cause staining	Wear eye protection	Wash from skin/eyes immediately using cold water	Low
Biohazard	Allergies; soil bacteria; contamination	Wash hands after use	Seek assistance	Low
Lamps	Temporary damage to eyes	Do not look directly at lamp	Wait for after image to disappear; seek appropriate assistance if needed	Low
Electrical appliances	Liquids near electrical appliances	Do not touch lamp/wires with wet hands; keep liquids away from lamp/wires	Seek assistance	Low





Graph

- Plot a graph of **absorbance** against **time** for each distance from the light.

Conclusion

- As the light intensity decreases, the rate of photosynthesis also **decreases**. This is because the lowered light intensity will **slow the rate of photoionisation** of the chlorophyll pigment, so the overall rate of the light dependent reaction will be slower.
- This means that less electrons are released by the chlorophyll, hence the **DCPIP accepts less electrons**. This means that it will **take longer to turn from blue to colourless**.
- When the DCPIP is **blue**, the **absorbance is higher**. The rate at which the absorbance decreases can therefore be used to determine the activity of the dehydrogenase enzyme. A **higher rate of decrease**, shown by a **steep gradient** on the graph, indicates that the **dehydrogenase is highly active**.

