

AQA Biology A-Level

Required Practical 1

Investigation into the effect of a named variable on the rate of an enzyme-controlled reaction.



The rate of reaction of an enzyme-controlled reaction is influenced by different factors: **the temperature, pH, concentration of the substrate, and the concentration of the enzyme.** The effect of each of these can be determined by **changing a single variable** and measuring its effect on the rate of reaction. It is important to **keep all other variables constant** so that they do not influence the results.

Equipment list

- Powdered milk suspension
- Trypsin solution (0.5%)
- Distilled water
- Hydrochloric acid (0.1M)
- 5cm³ syringes
- Flat bottomed tubes
- Water bath
- Timer

Method

In this method the named variable is temperature

1. Make two control samples:
 - Take two flat bottomed tubes.
 - Add 5cm³ of **milk suspension** to each tube.
 - Add 5cm³ of **distilled water** to one tube- this control will indicate the **absence of enzyme activity.**
 - Add 5cm³ of **hydrochloric acid** to the other- this control indicates the colour of a **completely hydrolysed sample.**
2. Take three test tubes and measure 5cm³ **milk** into each. Place in **water bath** at **10°C for 5 minutes** to equilibrate.
3. Add 5cm³ **trypsin** to each test tube simultaneously and **start the timer** immediately.
4. Record how long it takes for the **milk** samples to **completely hydrolyse** and become **colourless.**
5. Repeat steps 2-3 at temperatures of **20°C, 30°C, 40°C and 50°C.**
6. Find the **mean** time for the milk to be hydrolysed at **each temperature** and use this to work out the **rate of reaction.**

$$\text{Rate of reaction} = \frac{1}{\text{mean time}}$$





Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Broken glass	Cuts from sharp object	Take care when handling glass objects; keep away from edge of desk	Elevate cuts; apply pressure; do not remove glass from wound; seek medical assistance	Low
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
Hot liquids	Scalding	Handle with care; use tongs to remove boiling tubes from water bath; wear eye protection, keep away from the edge of the desk	Run burn under cold water; seek medical assistance	Low
Enzymes	Allergies	Avoid contact with skin/eyes; wear eye protection	Seek assistance	Low

Graph

- Plot a graph of **rate of reaction** against **temperature**.

Conclusion

- Milk contains a protein called **casein** which, when **broken down**, causes the milk to turn **colourless**. **Trypsin** is a **protease enzyme** which **hydrolyses the casein protein**.





- As the temperature increases from 10°C, **kinetic energy increases** so more **enzyme-substrate complexes** form. This means that the rate of reaction **increases** up to the optimum temperature.
- At temperatures beyond the optimum, **bonds** in the enzyme **tertiary structure break**, which changes the shape of the **active site**. This means that the substrate and enzyme are no longer **complementary**..

