MICROSCALE PHOSPHORYLASE

A SYNTHESIS ENZYME REACTION IN MINIATURE

Supports delivery of:

- National 4 Biology: Unit 1, Key Area 4 - Properties of enzymes (suggested learning activity includes experiments with phosphorylase).
- National 5 Biology: Unit 1, Key Area 4b - Enzymes (particularly as an example of a synthesis reaction).

Phosphorylase is an enzyme that catalyses the synthesis of starch from glucose-1-phosphate.

glucose-1-phosphate (substrate) → phosphorylase → **starch** (product)

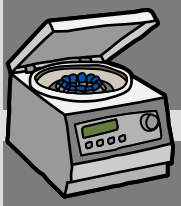
In this experiment, an enzyme extract is prepared from potato. Any starch is removed from the extract before it is incubated with fresh substrate for a specified time period. The synthesis of starch is determined using iodine, which will turn from yellow to blue-black in the presence of this product.

Technical notes:

- The enzyme extract should be prepared fresh from potato. This can be done by learners, using a blender, if appropriate in your setting. When fresh, the extract is cloudy and light in colour; over a 12 hour period, the extract will become brown, which interferes with clear detection of the iodine blue-black complex.
- Glucose-1-phosphate should be prepared fresh as it is unstable and will hydrolyse to glucose and phosphoric acid at room temperature. Can be stored in a fridge for up to 24 hours before use.

HEALTH & SAFETY

A risk assessment for this activity is available here. It is important to review and evaluate this model risk assessment within your own setting and class context.
Briefly, the significant hazards to consider in this experiment are:

HAZARD	RISK	CONTROL MEASURES
Centrifuge 	Injury to operator by spinning rotor or electrical fault.	Maintain centrifuge to good standard and ensure valid PAT certificate. Ensuring interlock lid design to prevent lid opening while rotor is turning.
Knife 	Risk of cuts and subsequent blood spill.	Careful classroom monitoring of knife use and ensuring all knives are collected in at the end of the lesson. Code of Practice for full control measures for blood spill.
 Blender	Injury to operator by spinning rotor or electrical fault.	Maintain centrifuge to good standard and ensure valid PAT certificate. Ensuring interlock lid design to prevent lid opening while blades are turning.
Iodine (0.005 mol/L) No significant hazard at this concentration.		

MATERIALS & METHOD

AIM

To investigate phosphorylase activity in potatoes.

INDEPENDENT VARIABLE

A range of variables will be discussed: incubation duration, substrate concentration, substrate type, enzyme concentration.

DEPENDENT VARIABLE

Phosphorylase activity, as determined by the colour of iodine following addition into the assay mixture.

MATERIALS REQUIRED - EXTRACTION

- 1 medium-sized potato (1 per class)
- Knife
- Small blender - this one is particularly good
- Water
- Muslin
- Centrifuge
- Centrifuge tubes
- Container to store enzyme extract
- Dimple tile
- Plastic pipette
- Iodine
- Water

MATERIALS REQUIRED - ASSAY

- $5cm^3$ 1% glucose-1-phosphate
- $5cm^3$ 1% glucose
- $5cm^3$ 1% maltose
- $5cm^3$ 1% sucrose
- $5cm^3$ phosphorylase extract
- Beaker of water
- Waterbath at 30°C
- 96-well microtitre plate
- 0.005 mol/L iodine solution
- $1cm^3$ plastic pipettes
- Marker pen
- Stopwatch

ENZYME EXTRACTION

STEP 1

Roughly chop 1 medium-sized potato into chunks and place into a blender. Add a small volume of water - just enough to make a pourable consistency.

STEP 2

Pour the pureed potato through a piece of muslin. Squeeze the contents of the muslin over a clean beaker.

STEP 3

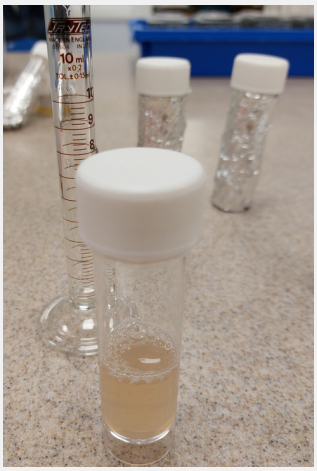
Divide the contents of the beaker between centrifuge tubes. Position the centrifuge tubes in the centrifuge rotor so the mass is balanced. Centrifuge at 6000 rpm for 5 minutes. A pellet will be visible in all tubes.

STEP 4

After centrifugation, use a plastic pipette to add a small sample of the supernatant to a dimple tile. Add a drop of iodine. If a blue-black colour appears, continue centrifugation until the supernatant is free from starch. If the iodine remains yellow, progress onto step 5.

STEP 5

Carefully transfer the supernatant from all centrifuge tubes to a clean container for storing the enzyme extract. Label the container "phosphorylase" and incubate at 30°C.



STEP 6

Collect the materials shown in the image. To avoid contamination, label the pipette bulbs with the solution to be used ("enzyme", "substrate", "water", "iodine").

If you are investigating the range of substrates that could be used in this reaction, additional pipettes will be required.

STEP 7 - EFFECT OF ENZYME CONCENTRATION

To investigate the effect of enzyme concentration, 4 wells will be required.

Add the solutions to each well using the plastic pipettes. The volumes are given as "number of drops".

DO NOT ADD IODINE YET! This will stop the reaction.

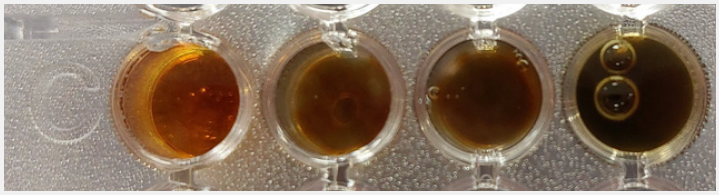
Reagent	Volume (number of drops)			
	Well 1	Well 2	Well 3	Well 4
Water	4	2	1	0
Substrate	2	2	2	2
Enzyme	0	2	3	4
Iodine	1	1	1	1
TOTAL	7	7	7	7

STEP 7 CONT...

Float the 96-well plate in the waterbath and leave for 20 minutes.

After 20 minutes, add 1 drop of iodine to each of the wells and note the colour of the iodine. The darker the colour, the more starch is present, showing a fast rate of synthesis reaction.

increasing enzyme concentration



STEP 8 - EFFECT OF SUBSTRATE CONCENTRATION

To investigate the effect of substrate concentration, 4 wells will be required. Add the solutions to each well using the plastic pipettes. The volumes is given as "number of drops".
DO NOT ADD IODINE YET! This will stop the reaction.

Reagent	Volume (number of drops)			
	Well 1	Well 2	Well 3	Well 4
Water	4	2	1	0
Substrate	0	2	3	4
Enzyme	2	2	2	2
Iodine	1	1	1	1
TOTAL	7	7	7	7

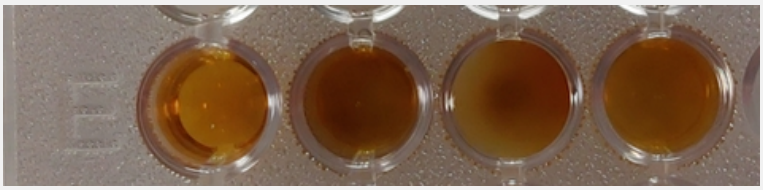
Float the 96-well plate in the waterbath and leave for 20 minutes.

After 20 minutes, add 1 drop of iodine to each of the wells and note the colour of the iodine. The darker the colour, the more starch is present, showing a fast rate of synthesis reaction.

STEP 8 CONT...LIKELY RESULTS

After 20 minutes, under these conditions, very minimal starch production was observed. Too little enzyme was present to allow the reaction to proceed.

increasing substrate concentration



The protocol could be adapted to include 4 drops of enzyme in each well (as shown in the table below). However, the maximum number of drops in these wells is 7 so an alternative vessel would need to be used (e.g. a 24-well microtitre plate or dimple tile).

Reagent	Volume (number of drops)			
	Well 1	Well 2	Well 3	Well 4
Water	4	2	1	0
Substrate	0	2	3	4
Enzyme	4	4	4	4
Iodine	1	1	1	1
TOTAL	9	9	9	9

STEP 9 - EFFECT OF INCUBATION TIME

To investigate the effect of incubation time on phosphorylase activity, 4 wells will be required. Add the solutions to each well using the plastic pipettes. The volumes is given as "number of drops". DO NOT ADD IODINE YET! This will stop the reaction.

STEP 9 CONT...

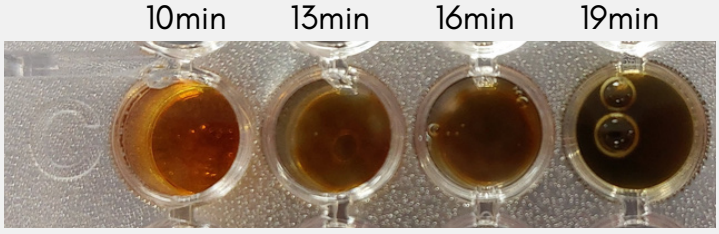
Reagent	Volume (number of drops)			
	Well 1	Well 2	Well 3	Well 4
Substrate	2	2	2	2
Enzyme	4	4	4	4
Iodine	1	1	1	1
TOTAL	7	7	7	7

Float the 96-well plate in the waterbath.

- After 10 minutes, add 1 drop of iodine to well 1.
- After 13 minutes, add 1 drop of iodine to well 2.
- After 16 minutes, add 1 drop of iodine to well 3.
- After 19 minutes, add 1 drop of iodine to well 4.

Note the colour of the iodine. The darker the colour, the higher the concentration of starch present.

reactions stopped after (left to right)...



Adaptations:
Substitute 96-well microtitre plates for the base of a petri dish. Draw 4 circles using a permanent marker pen on the external side of the petri dish base. Add the drops of the different reagents within each circle. The petri dish base will float on water.

Adaptations cont.

Prefer not to have pupils crowding around a couple of waterbaths? This will work well at room temperature. Alternatively, pupils could have their own mini-waterbath by filling an old ice-cream tub with warm water and monitor the temperature using a thermometer.