PROTOCOL

INVESTIGATING PHOSPHATASE ACTIVITY

Extract phosphatase from bean sprouts and catalyse a degradation reaction

sserc

Page

Background

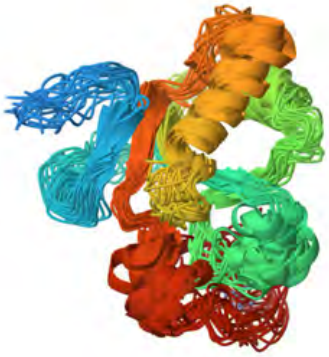
Phosphatase enzymes are involved in a range of metabolic reactions. A key function of these enzymes is to release phosphate groups into the metabolic pool thereby increasing their availability for use in a range of processes including ATP synthesis and membrane construction.

Acid phosphatases (those with an optimum pH <7.0) can be extracted from a range of plant tissues – germinating mung beans or bean sprouts are a cheap and reliable source. The substrate is phenolphthalein bisphosphate (PPP). Under suitable conditions, phosphatase catalyses the breakdown of PPP to form phenolphthalein (PP) (Figure 1).

At neutral or acidic pH, the products of this reaction (PP and phosphate) are both colourless – so their presence is difficult to detect. This can be overcome through the addition of sodium carbonate which has 2 effects:

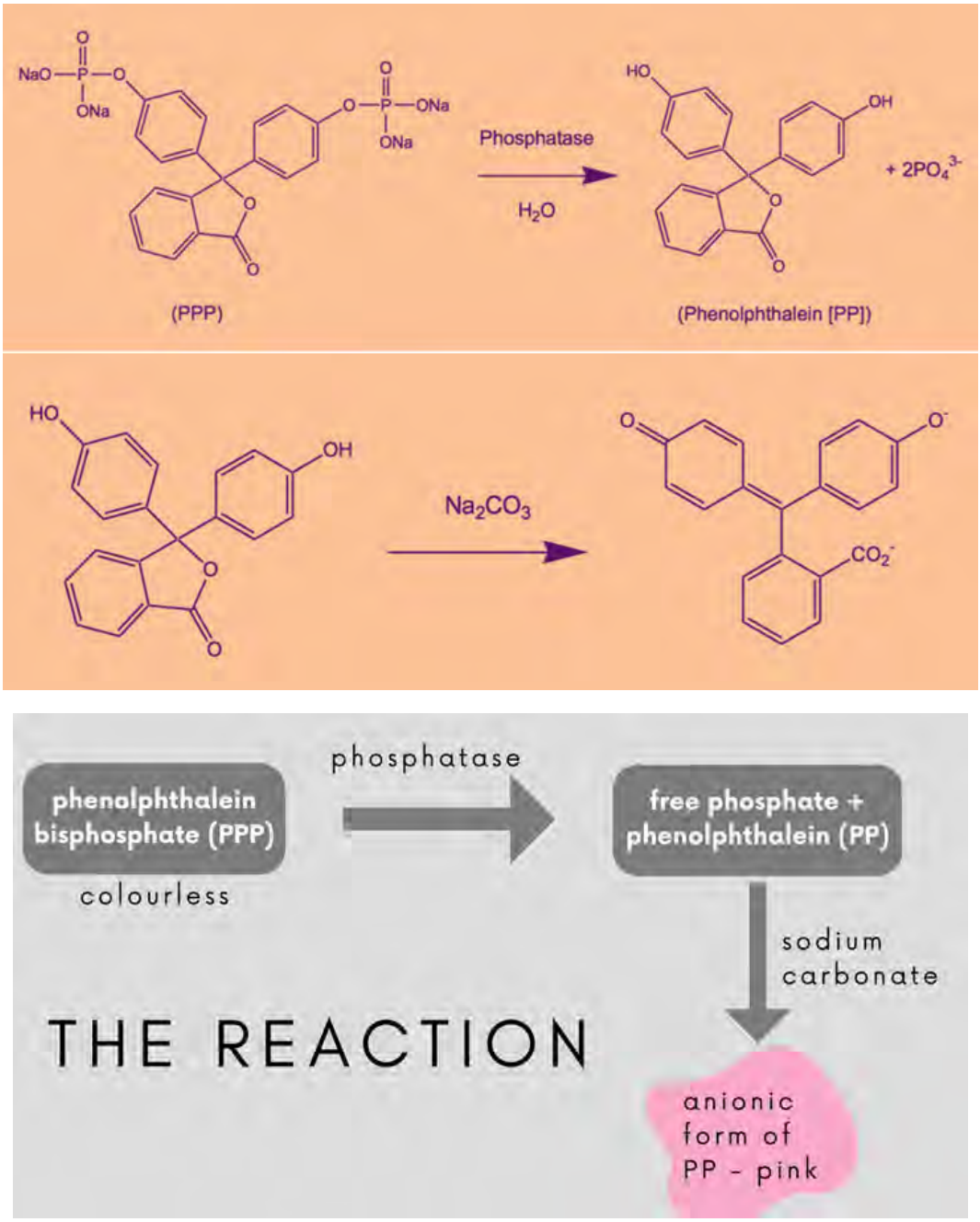
1) raising the pH of the solution >pH10 with cessation of enzyme activity,

2) converts PP to its anionic form, which is pink.



NMR structure of a phosphatase enzyme from Arabidopsis thaliana

Figure 1: Phosphatase-catalysed breakdown of PPP to form free phosphate and PP, both of which are colourless. The addition of sodium carbonate stops further enzyme activity and converts PP to its anionic, pink form.



AIM

To investigate the effect of pH on phosphatase activity in bean sprouts.

This protocol can be adapted to investigate a range of independent variables, including:

- effect of temperature on phosphatase activity
- effect of enzyme concentration on phosphatase activity
- effect of substrate concentration on phosphatase activity
- effect of end-product inhibition on phosphatase activity
- effect of tissue type on phosphatase activity.

RISK ASSESSMENT

A risk assessment for this activity can be downloaded from the SSERC website. Click here. This should be adapted for your centre, where appropriate.

Briefly, the main hazard associated with this protocol is the use of a centrifuge. This should be PAT tested and care must be taken to ensure the lid cannot be opened while the rotor is spinning. The centrifuge tubes must be accurately balanced in the rotor.

MATERIALS REQUIRED PER PAIR

Part 1 - Preparation of the enzyme extract

- 20 g bean sprouts
- pestle and mortar
- water
- 3 cm^3 plastic pipettes
- microfuge
- 6x microfuge tubes
- marker pen
- container to store enzyme extract

Part 2 - Phosphatase Assay

- enzyme extract
- 25 cm^3 10% (w/v) sodium carbonate
- stopwatch
- access to a waterbath (30°C)
- paper towels
- 14x absorption cuvettes
- colorimeter (550 nm)
- cuvette rack
- 10 cm^3 citric acid / phosphate buffer (pH 5.0)
- 10 cm^3 citric acid / phosphate buffer (pH 7.0)
- 6 cm^3 0.2% phenolphthalein phosphate
- 1 cm^3 automatic pipette and tips
- polystryrene cup

OVERVIEW OF METHOD

1. Crush 20 g bean sprouts in a mortar with 5 cm^3 water.

2. Divide extract between 6 microfuge tubes.

3. Centrifuge for 5 minutes. Store supernatant for next step.

4. Incubate buffer, enzyme and substrate at 30 °C.

5. Add 1 cm^3 sodium carbonate to 7 cuvettes.

6. Mix 2 cm^3 enzyme, 10 cm^3 buffer, 2 cm^3 substrate. Every 2 minutes, transfer 1 cm^3 to a cuvette

STEP-BY-STEP METHOD

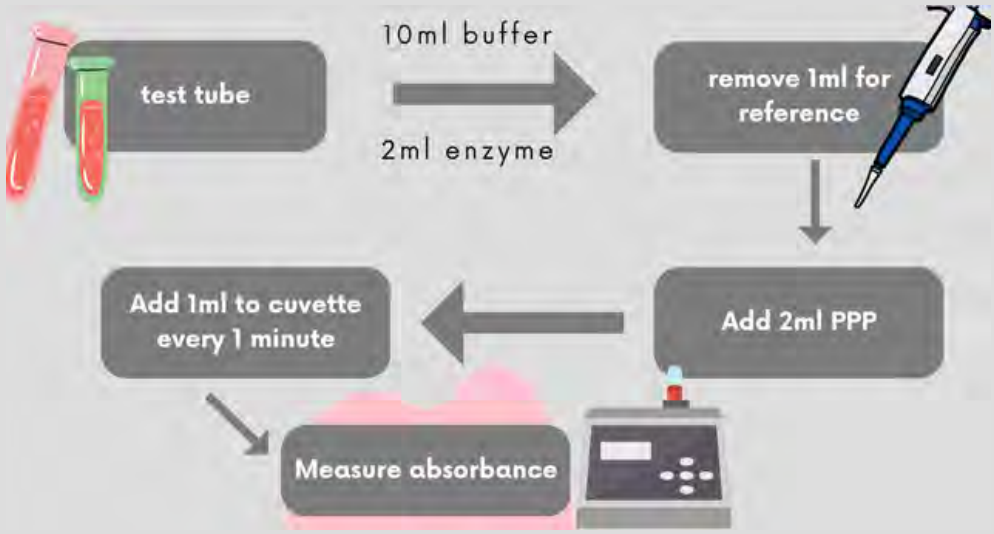
Part 1 - Preparation of the enzyme extract

1. Place 20 g bean sprouts in a mortar. Remove and discard the green testa (seed case) if it is attached. Germinated mung beans can also be used - use about 30 mung beans per extraction.
2. Add 5 cm^3 water to the mortar. Grind the bean sprouts with a pestle to achieve a smooth paste.
3. Cut the tip off a plastic pipette. Divide the extract equally between 6 microfuge tubes (should be approximately equal volume to ensure the centrifuge rotor is balanced).
4. Centrifuge the samples for 5 minutes. Using a plastic pipette, transfer the supernatant from the microfuge tubes to a labelled container.

To carry out the assay at pH 5 and pH 7, at least 5 cm^3 enzyme extract is required.

Part 2 - Phosphatase Assay

The following diagram gives an overview of this part of the protocol.



All solutions should be kept at 30 °C throughout the assay. It is accurate to dispense the volumes outlined in the following steps using an automatic 1 cm^3 pipette with a clean tip.

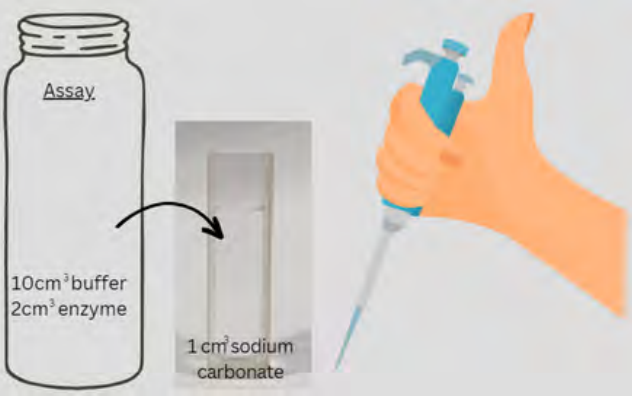
1 Transfer water from the waterbath (30 °C) to the polystyrene cup. Stand the container of buffer (pH 5), enzyme extract and substrate (PPP) in the polystyrene cup. This provides each learner with their own individual waterbath. If carrying this out for an assessed piece of work, a thermostatically-controlled waterbath should be used instead.

In the image opposite, two polystyrene cups have been used, with water at 30 °C. The substrate and enzyme are stored in bijou bottles in one cup (left), while the buffer is held in a universal bottle in the second cup (right).

2 Using the 1 cm^3 automatic pipette, add 1 cm^3 sodium carbonate into 7 cuvettes. Discard the tip.

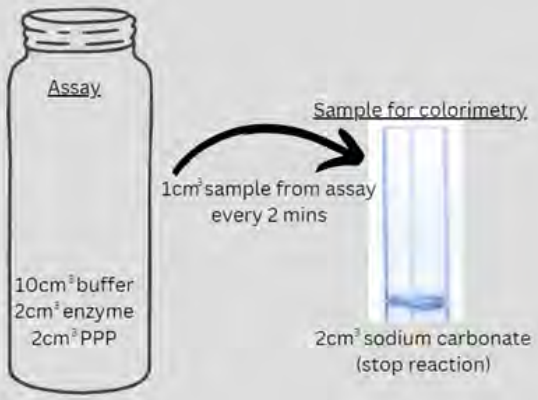
3 Transfer 2 cm^3 enzyme extract to 10 cm^3 buffer solution.

4 For the colorimetric blank: transfer 1 cm^3 of the mixture from the container of buffer + enzyme (step 3) to the first cuvette (containing sodium carbonate).



5 Transfer 2 cm^3 PPP to the enzyme/buffer mixture. Start the stopwatch. Mix the contents thoroughly but without creating too many bubbles.

6 At 2 minute intervals, transfer 1 cm^3 of the PPP/ enzyme / buffer mixture to a cuvette. The sodium carbonate will stop the reaction and convert the product (PP) to its anionic form, which is pink.



7 Use the colorimetric blank (step 4) to zero the colorimeter. This contains buffer and enzyme only and is held in cuvette 1.

Measure the absorbance of the remaining solutions at 550 nm. If using the Mystrica colorimeter, this will require using the green diode.

The assay can now be repeated using the buffer at pH 7.0.

RESULTS

Present results in a table similar to the one opposite.

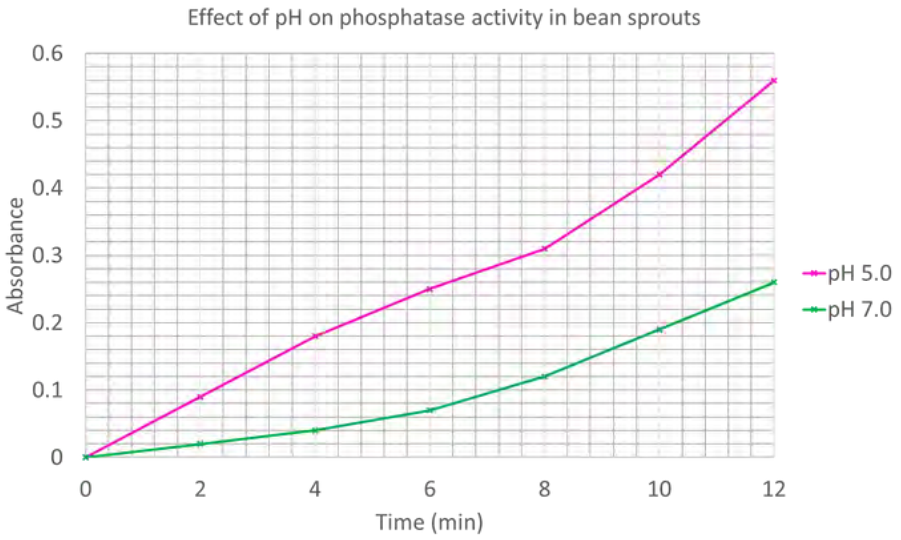
The results can be then plotted as a line graph.

Sample results are shown on the following page.

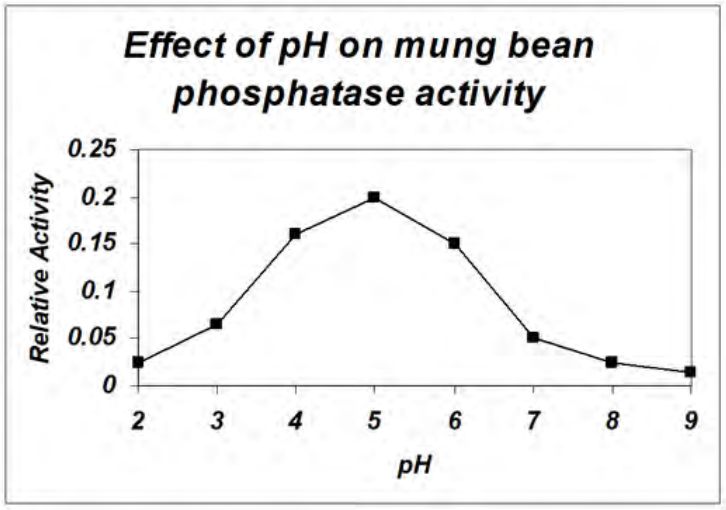
Time (min)	Absorbance	
	pH 5.0	pH 7.0
0		
2		
4		
6		
8		
10		
12		

SAMPLE RESULTS

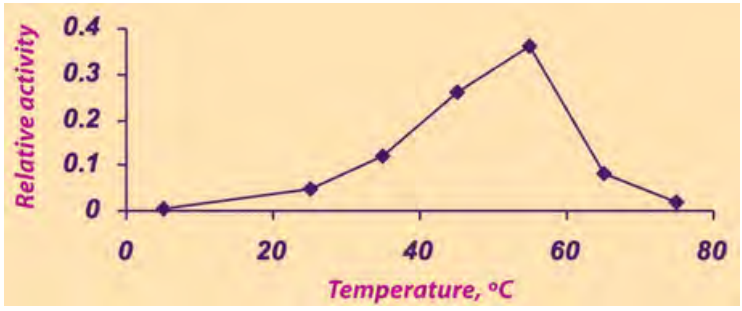
Time (min)	Absorbance	
	pH 5.0	pH 7.0
0	0.00	0.00
2	0.09	0.02
4	0.18	0.04
6	0.22	0.07
8	0.31	0.12
10	0.42	0.19
12	0.56	0.26



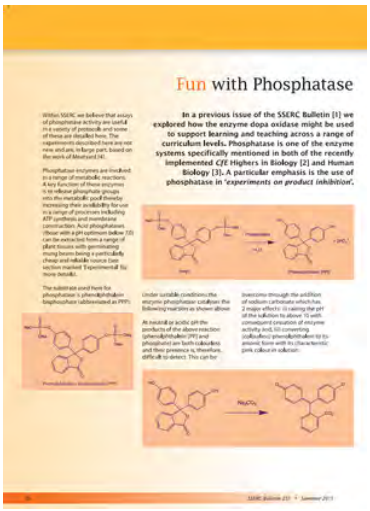
When this experiment is carried out over a range of pH values, the following activity is observed (right). This experiment involved taking a fixed time-point measurement after 10 minutes at each pH value, showing an alternative approach to this protocol.



This chart (right) presents expected results from the protocol when investigating temperature as the independent variable.

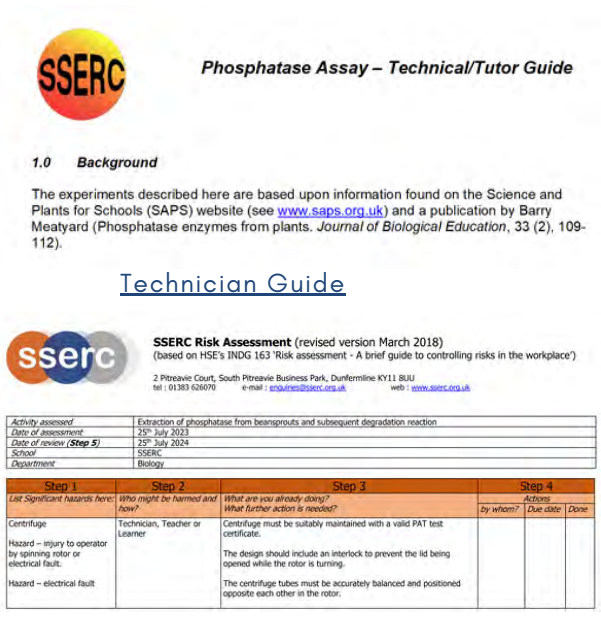


SUPPLEMENTARY RESOURCES


Fun with Phosphatase

In a previous issue of the SSERC Bulletin [1] we explored how the enzyme dopa oxidase might be used to support learning and teaching across a range of curriculum levels. Phosphatase is one of the enzyme systems specifically mentioned in both of the recently implemented CfE Highers in Biology [2] and Human Biology [3]. A particular emphasis is the use of phosphatase in 'experiments on product inhibition'.

SSERC bulletin (2015) available to download.


Phosphatase Assay – Technical/Tutor Guide

1.0 Background

The experiments described here are based upon information found on the Science and Plants for Schools (SAPS) website (see www.saps.org.uk) and a publication by Barry Meatyard (Phosphatase enzymes from plants. *Journal of Biological Education*, 33 (2), 109-112).

Technician Guide

SSERC Risk Assessment (revised version March 2018)
(based on HSE's INDG 163 'Risk assessment - A brief guide to controlling risks in the workplace')

Activity assessed	Extraction of phosphatase from beansprouts and subsequent degradation reaction
Date of assessment	25th July 2023
Date of review (Step 5)	25th July 2024
School	SSERC
Department	Biology

Step 1	Step 2	Step 3	Step 4		
List Significant hazards here:	Who might be harmed and how?	What are you already doing? What further action is needed?	Actions by whom?	Due date	Done
Centrifuge Hazard – injury to operator by spinning rotor or electrical fault. Hazard – electrical fault	Technician, Teacher or Learner	Centrifuge must be suitably maintained with a valid PAT test certificate. The design should include an interlock to prevent the lid being opened while the rotor is turning. The centrifuge tubes must be accurately balanced and positioned opposite each other in the rotor.			

Risk Assessment

PPT to download for lessons