Following the normal protocol, this should imitate the results of investigating the effect of phosphates on phosphatase experiment well. Care should be taken to ensure that lids for sodium carbonate bottles are not put on any buffer bottles by accident, as this will trigger the phenolphthalein to turn pink.

Preparation of buffers for PPP experiment alternative are as follows:-

Phenolphthalein prepared as follows- Dissolve 0.5 g in 50 cm^3 of ethanol (FLAMMABLE) and make up to 100 cm^3 with water. Label the container as FLAMMABLE.

Phosphate Buffer (pH5) 0M- 20 drops of Phenolphthalein in 150ml water.

Phosphate Buffer (pH5) 0.05M- 10 drops of Phenolphthalein in 150ml water.

Phosphate Buffer (pH5) 0.10M- 5 drops of Phenolphthalein in 150ml water.

Phosphate Buffer (pH5) 0.20M- 2 drops of Phenolphthalein in 150ml water.

Phosphate Buffer (pH5) 0.30M- 1 drop of Phenolphthalein in 150ml water.

If using negative control add distilled water with **no** phenolphthalein.

Sodium carbonate prepared as normal.

PPP prepared as **water**.

Phosphatase extraction carried out as normal to simulate proper experiment.

Photo of expected results:-

0M 0.05M 0.1M 0.2M 0.3M

Science &Plants for Schools

Student Sheet 14

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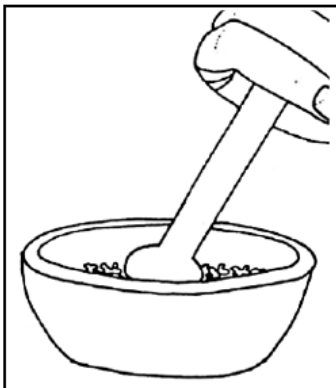
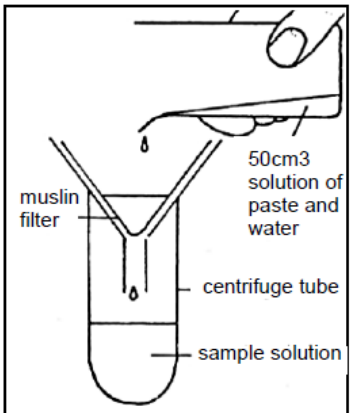
Phosphatase Enzymes in Plants

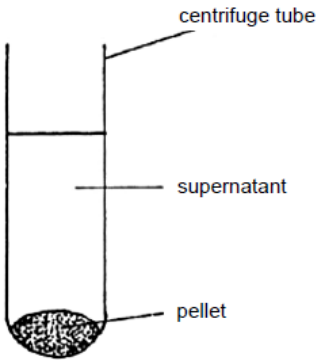
Read these instructions carefully before you start.



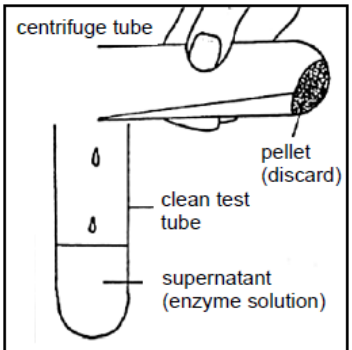
1. Grind approximately 50 five-day-old mung bean seedlings to a paste with a few drops of water.



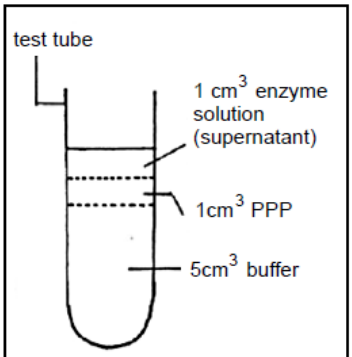
2. Make the paste up to 50 cm3 with distilled water. Then filter through 2 layers of muslin into a clean centrifuge tube.



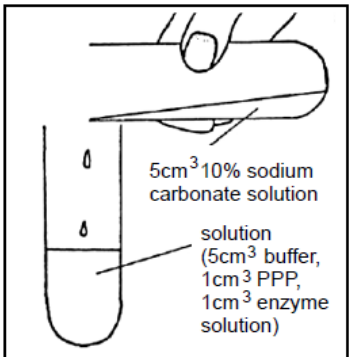
3. Centrifuge at high speed in a bench centrifuge for about 5 minutes to produce a tight pellet.



4.Carefully pour the supernatant into a clean tube. This will be used as the enzyme solution. Discard the pellet.



5. Accurately measure 5 cm3 of buffer, 1 cm3 of PPP (1% in water) and 1 cm3 of enzyme solution into a test tube. Add the enzyme solution last. Mix well.
Incubate for 20 minutes at 30°C.



6. Add 5 cm3 of 10% sodium carbonate solution and invert the tube once to mix.

In step 5, the following reaction occurs:

phenolphthalein phosphate ----------(phosphatase enzyme)----------> free phenolphthalein + phosphate

7. The addition of sodium carbonate solution stops the reaction and, if free phenolphthalein is present, a pink colour is seen.
8. Measure the pink colour in a colorimeter using a blank with 1cm3 water in place of the supernatant. If you have no colorimeter, you can compare your samples with a serial dilution of lab phenolphthalein, to give you an approximate estimate of the amount of phenolphthalein released by the enzyme.