

The influence of pH, temperature and activators on the activity of salivary amylase

Task 1. Determination of the achromatic point

The aim of this activity is to determine the activity of salivary amylase.

In the first stage you should establish an adequate concentration of the saliva to achieve the achromatic point within 3-15 minutes. For this purpose add 1 cm³ of saliva to the graduated cylinder, dilute it with distilled water (1:100 or a 0.1% enzyme solution) and mix precisely.

Achromatic point is defined as the time at which the products from the hydrolysis of starch are released and the color of iodine-starch complex is no longer visible.

Example: Achromatic point is 8 min, as shown below:



Procedure:

Add 3 drops of 0.001 mol/dm³ I₂ in KI (iodine solution) and 1 drop of 2 mol/dm³ HCl to approx. 15 wells of the plate.

Add 5 cm³ of 1% starch solution, 2 cm³ of 1% NaCl and 2 cm³ of phosphate buffer solution (pH=6.6) to a test tube and mix.

Incubate the test tube for 5 minutes in water bath (37°C). After that, add 1 cm³ of prepared enzyme solution (diluted saliva), leaving the tube in a bath. The moment of mixing enzyme with substrate starts the enzymatic reaction and this is the zero time.

Exactly after 1 minute of incubation transfer 2 drops of incubation mixture to the first well containing the iodine solution. From this point on continue this procedure at one minute intervals until the achromatic point is reached. Observe color changes and note the time corresponding to the achromatic point. In this point the well will have the same color as iodine without starch (yellow).

In case of too short time (less than 3 minutes) or too long



(more than 15 minutes), repeat the test with another dilution of saliva.

Task 2. Evaluation of the effect of pH on the amylase activity

The aim of this activity is to evaluate the influence of pH on the amylase activity.

Procedure:

Add 3 drops of 0.001 mol/dm³ I₂ in KI (iodine solution) and 1 drop of 2 mol/dm³ HCl to the wells of the plate, creating 3 rows.

Prepare 3 test tubes (marked as 1, 2 and 3). Add 5 cm³ of 1% starch solution and 2 cm³ of 1% NaCl to each of them.

Next, add the following solutions to 3 test tubes:

1st test tube: 2cm³ of 0.2 mol/dm³ phosphate buffer pH=6.6

2nd test tube: 2cm³ of 0.2 mol/dm³ phosphate buffer pH=8.0

3rd test tube: 2cm³ of 0.2 mol/dm³ phosphate buffer pH=5.0

Incubate the test tubes for 5 minutes in water bath (37°C). After that, add 1 cm³ of prepared enzyme solution (diluted saliva), leaving the tubes in a bath.

Exactly at one minute intervals transfer 2 drops of incubation mixture (do not take out the tubes from a bath) to the wells containing the iodine solution, to receive 3 rows with 3 different pH values.

Observe color changes and note the time corresponding to the achromatic point for each pH value.

Task 3. Evaluation of the effect of chloride ions on the amylase activity

The aim of this activity is to evaluate the influence of chloride ions on the amylase activity.

Procedure:

Add 3 drops of 0.001 mol/dm³ I₂ in KI (iodine solution) and 1 drop of 2 mol/dm³ HCl to the wells of the plate, creating 2 rows.

Prepare 2 test tubes (marked as 1 and 2). Add 5 cm³ of 1% starch solution and 2 cm³ of 0.2 mol/dm³ phosphate buffer of optimum pH (the result from previous experiment).

Next, add:

- to the test tube 1: 2cm³ of 1% NaCl
- to the test tube 2: 2cm³ of distilled water

Put both tubes into a water bath (37 °C) for 5 minutes.



Next, add 1 cm³ of saliva (source of enzyme). Accurately every 1 minute take 2 drops of the incubate from each tube (1 and 2) and add into the wells of the previously prepared porcelain/plastic plate - to obtain two rows with two different solutions on the plate.

Observe the color changes and note the achromatic point for both samples.

Task 4. Evaluation of the effect of the temperature on the amylase activity

The aim of this activity is to evaluate the influence of temperature on the amylase activity.

Procedure:

Add 3 drops of 0.001 mol/dm³ I₂ in KI and 1 drop of 2 mol/dm³ HCl to the wells of porcelain/plastic plate.

Prepare 3 test tubes (marked as: 1,2,3) with 5 cm³ of 1% starch solution, 2 cm³ of 1% NaCl and 2 cm³ of 0.2 mol/dm³ phosphate buffer of optimum pH (the result of Task 2).

- test tube 1: put into water bath (temp. 37°C)
- test tube 2: leave at room temperature
- test tube 3: keep on ice

After 5 minutes add 1 cm³ of saliva solution to each tube (1,2,3).

Accurately every 1 minute take 2 drops of the incubate from each tube (1,2,3) and add into the wells of the previously prepared porcelain/plastic plate - to obtain three rows with three different temperature values.

Observe the color changes and note the achromatic point for each temperature value.

