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Assay of

Salivary Amylase

enzyme activity

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ENZYME PROTOCOLS

Assay of Salivary Amylase enzyme activity

Aim:

To determine activity of Amylase in Saliva

Principle:

Amylase is the hydrolytic enzyme which breaks down many polysaccharides like Starch, Amylose, dextrins and yields a disaccharide i.e., Maltose.

$$(C_6H_{10}O_5)n + H2O \rightarrow n(C_{12}H_{22}O_{11})$$

Reagents:

1. **Substrate (Starch) :** Mix 1 gm of soluble starch in 200ml of 0.1M Phosphate buffer (pH 6.8) boil for 3 minutes and cool to room temperature and filter it necessary.
2. **Enzyme:** Saliva is the best and easily available source of amylase. Collect some saliva in a beaker and dilute it to 1:20 dilution with distilled water.
3. **1% Sodium chloride:** It is necessary for enzyme activity
4. **DNS (Dinitro Salicylic acid):** Dissolve 1.6 gm of NaOH in 20ml of distilled water. Take 1gm of 3,5 DNS in NaOH solution. In other beaker take 30gm of Sodium potassium tartarate. Dissolve in 50ml of distilled water. Mix this DNS solution and finally make the volume up to 100ml with distilled water.
5. **Standard solution of Maltose:** It is prepared by dissolving 200mg Maltose in 100ml of water (2mg / 1ml).

Procedure:

Take 0.5ml of substrate and 0.2ml of 1% NaCl in a test tube and pre-incubate at 37^0C for 10 minutes then add 0.3ml of dilute saliva and incubate for 15 minutes at 37^0C. Stop the reaction by addition of 1 ml of DNS reagent mix well and keep the test tubes in boiling water both for 10 minutes. Cool and dilute with 10ml of distilled water. Read the colour developed at 520 nm. Simultaneously setup the colour developed at 520nm. Simultaneously setup the blank as per the test by adding DNS prior to the addition of enzyme simultaneously. Setup the standards of different test tubes and repeat the experiment as per the test and measure the colour developed at 520nm absorbance.

Preparation of Phosphate buffer:

Dissolve 0.2M (2.7218 grams) of KH2PO4 in 100ml of distilled water to this solution add 0.5M (2.8053 grams) KOH drop by drop till the pH is set to 6.8. Then make it to 200ml with distilled water. So the final concentration is 0.1M of 200ml Phosphate buffer.

Result:

The amount of Maltose in the given unknown sample is _____ gms of Maltose formed per 100ml of enzyme per one hour.

S.No.	Volume of Std (ml)	Volume of Distilled water (ml)	Concentration of Standard (ml)	Volume of DNS (ml)	Boiling water bath for 10 minutes and cool it	Volume of Distilled water (ml)	OD at 520 nm
1	Blank	1.0	0.0	1.0		10	
2	0.2	0.8	0.4	1.0		10	
3	0.4	0.6	0.8	1.0		10	
4	0.6	0.4	1.2	1.0		10	
5	0.8	0.2	1.6	1.0		10	
6	1.0	0.0	2.0	1.0		10	

Test OD – Blank OD = _____ = _____

Calculation:

1.5 mg of Maltose formed / 0.3. ml / 15 minutes

1.5 X 4 mg of Maltose formed / 0.3 ml of Enzyme / 1 hour

1.5 X 4 X 3.3 mg of Maltose formed / 1ml of Enzyme / 1 hour

1.5 X 4 X 3.3 X 100 mg of Maltose formed / 100ml of Enzyme / 1 hour