HiPer® Yeast Cell Immobilization Teaching Kit

Product Code: HTBC001

Number of experiments that can be performed: 10

Duration of Experiment: 3 days

Day 1- Preparation of solution, media and reviving the yeast strain: 2 hours
Day 2- Inoculation: 15 minutes
Day 3- Protocol: 3 hours

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store Yeast cells, 1.5% Calcium chloride solution, Glucose standard (1mg/ml) and Diluent Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

Index

Aim:

To learn the technique of immobilizing yeast cells in alginate beads and determine its enzymatic activity by invertase assay.

Introduction:

Immobilization is a technique for the combination of a biocatalyst in an insoluble support matrix. The matrix is usually a high molecular weight polymer such as polyacrylamide, starch, cellulose, etc. The advantage of immobilizing enzymes or cells over free cells is to increase their stability and efficiency. The immobilized enzymes or cells can also be recovered at the end of the reaction and can be used repeatedly.

Principle:

In 1916 two scientists named Nelson and Griffin discovered that invertase (an enzyme) shows the same activity when absorbed on a solid surface as when uniformly distributed throughout the solution. This was the first discovery of enzyme immobilization technique. An enzyme is usually immobilized onto an inert, insoluble material e.g. Calcium alginate. This is produced by the reaction of a mixture of Sodium alginate solution with Calcium chloride. These beads provide increased resistance to changes in conditions such as pH or temperature. They also allow enzymes to be held in place throughout a reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so are widely used in industry for enzyme catalyzed reactions.

Whole cell immobilization is an alternative to enzyme immobilization. Basically, immobilization of live cells is very similar to the enzyme counterpart. In the past, various cells have been immobilized: bacteria, yeasts, fungi, plant tissues, mammalian tissues, and insect tissues. Once the cells are immobilized, the cell viability must be concomitantly sustained over a long period of time.

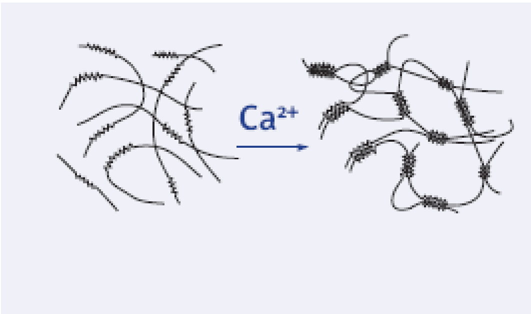


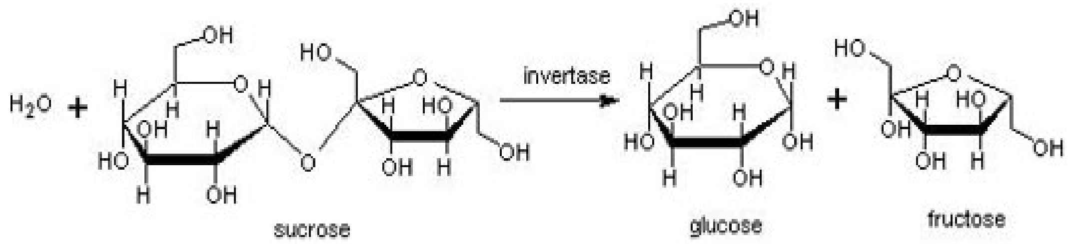
Fig1: The alginate is ionically cross-linked by the calcium ions

The lower microorganisms (bacteria, yeasts, and fungi) can be easily immobilized with a number of methods: entrapment, ion exchange adsorption, porous ceramics, and even covalent bonding. Most of the principles involved in enzyme immobilization are directly applicable to cell immobilization.
There are five methods for immobilization of enzymes or cells.

1. **Adsorption:** It is a method which involves electrostatic interaction such as Van der waals forces, ionic and hydrogen bonding between the enzymes or cells and the support matrix.
2. **Covalent Binding:** This method involves formation of covalent bonds between the enzymes or cells and the support matrix. The bond is normally formed between the functional groups present on the surface of the support and functional groups belonging to amino acid residues on the surface of the enzyme.
3. **Entrapment:** In this method the enzyme molecules are mixed with a polyionic polymer material and then crosslinking of the polymer with multivalent cations in an ion exchange reaction to form a lattice structure that traps the enzymes or cells.

4. **Encapsulation:** This can be achieved by enveloping the enzymes or cells within various forms of semipermeable membranes.
5. **Crosslinking**: This involves joining of enzymes or cells with each other to form large three-dimensional complex structures and can be achieved by physical or chemical methods without any support system.

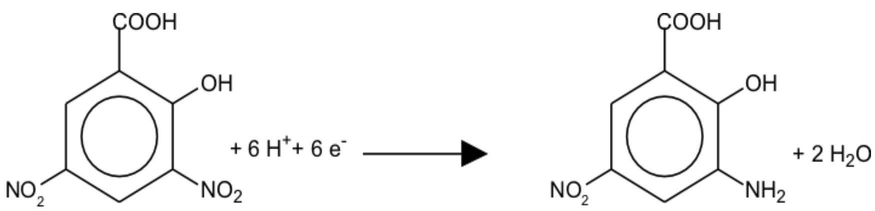
The immobilized yeast cells can be used for the production of several enzymes namely invertase, zymase, lactase, maltase etc. Invertase/ Sucrase hydrolyzes sucrose into glucose and fructose as shown in the following reaction.



Disaccharide **Monosaccharides**

The action of invertase from yeast cells can be shown by growing yeast cells or entrapped (in beads) yeast cells. Beads containing immobilized yeast will convert sucrose into the reducing sugars glucose and fructose that can be detected using DNSA.

3, 5-Dinitrosalicylic acid (DNSA) is used extensively in biochemistry for the estimation of reducing sugars. It detects the presence of free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose). During this reaction DNSA is reduced to 3- amino- 5-nitrosalicylic acid (ANSA) which under alkaline conditions is converted to a reddish brown coloured complex which has an absorbance maximum of 540 nm.



3, 5 Dinitrosalicylic acid (DNSA) **3- amino- 5 Nitrosalicylic acid (ANSA)**

Fig 2: Chemical reaction for DNSA method

Kit Contents:

The kit demonstrates the immobilization of yeast cells within alginate beads.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity 10 expts	Storage
1	TKC112	Yeast Cells	1 No.	2-8 °C
2	MB114	Sodium alginate	10 g	R T
3	TKC194	1.5% Calcium chloride solution	550 ml	2-8 °C
4	M1363	Yeast Extract Peptone Dextrose Broth	40 g	R T
5	MB053	Agar Powder, Bacteriological	3.5 g	R T
6	TKC265	Glucose standard (1 mg/ml)	6.6 ml	2-8 °C
7	TKC266	Diluent Buffer	60 ml	2-8 °C
8	GRM601	Sucrose	1.5 g	RT
9	TKC240	DNSA Reagent	60 ml	RT
10	GRM598	Potassium sodium tartrate, tetrahydrate	21 g	RT
11	PW143	Centrifuge Tubes (50 ml)	2 Nos.	R T

Materials Required But Not Provided:

Glass wares: Measuring cylinder, Beaker, Conical flasks, Test tubes
Reagents: Distilled Water*
Other requirements: Tips, Crushed ice, 30°C Incubator, 30°C Incubator shaker, Sterile loop, Pipette, Tips, Strainer, Boiling water bath, Spectrophotometer, Petri plates, Test tube stand

Storage:

HiPer® Yeast Cell Immobilization Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store the Yeast cells, Calcium chloride solution, Glucose standard (1mg/ml) and Diluent Buffer at 2-8 °C. Other kit contents can be stored at room temperature.

Important Instructions:

1. Read the entire experiment carefully before starting the experiment.
2. The assay should be carried out at the same time and in the same buffer conditions.
3. **Preparation of YPD Agar plates (20 ml):** Suspend 1 g of YPD Broth and 0.3 g of agar in 20ml of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes and pour on a sterile petri plate.
4. **Preparation of YPD broth (50 ml):** Suspend 2.5 grams of YPD Broth in 50ml of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
5. **Preparation of 4% Sodium alginate solution (50 ml):** Suspend 2 grams of Sodium alginate in 50 ml of distilled water*. Heat to dissolve and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
6. **Preparation of 40% Potassium sodium tartrate (Rochelle's salt) (50 ml):** Dissolve 20 g of Potassium sodium tartrate in 25 ml of distilled water and make up the volume to 50 ml.
7. **Preparation of Diluent Buffer containing 2.5% sucrose (5 ml):** Dissolve 0.125 g of sucrose in 2.5 ml of diluent buffer and make up the volume to 5 ml.
8. Always use clean glassware for the experiment.

***Recommended Product for use: ML064 – Molecular Biology Grade Water**

Procedure:

Day 1:

1. Open the vial containing lyophilized culture and resuspend the cells with 0.25 ml of YPD broth.
2. Streak a loopful of culture from the lyophilized vial on to YPD Agar plate.
3. Incubate the plate overnight at 30^oC.

Day 2:

1. Inoculate a single colony from the revived plate in 50 ml of YPD broth.
2. Incubate at 30^oC shaker overnight at 200 rpm.

Day 3:

A] Yeast Cell Immobilization:

1. In a 50 ml centrifuge tube, take 20 ml of the yeast culture and to it add 20 ml of the 4% Sodium alginate solution. Put the cap tightly.
2. Mix the culture with Sodium alginate solution properly.
3. Take 50 ml of 1.5% Calcium chloride solution in a conical flask. Crush the ice and keep the flask on ice.
4. With a 1 ml pipette, take the alginate and yeast culture mix and add drop wise to the Calcium chloride solution. While adding make sure that the flask is swirled gently.
5. Leave the immobilized yeast cell beads to harden in the Calcium chloride solution for 5–10 minutes. The alginate will be ionically cross-linked by the calcium ions.
6. Isolate the beads after discarding the solution.

B] Detection of Enzymatic Activity of Immobilized Cells by Invertase Assay:

1. Take 0.5 g of beads in a test tube and wash with 2 ml of diluent buffer containing 2.5 % sucrose and drain. Add 2 ml of diluent buffer containing 2.5 % sucrose.
2. Take five clean test tubes and label them as I – V. Take aliquots of 200 µl at 0, 30, 60, 90 and 120 minutes in the labeled test tubes. Dilute these aliquots fivefold (e.g. 40 µl aliquot and 160 µl diluent buffer) and estimate the presence of glucose by following the protocol of standard graph preparation.

DNSA Method for Standard Graph Preparation:

1. Take six tubes and label them as Blank and 1 - 5.
2. Make dilutions of glucose standards with concentrations of 40, 80, 120, 160 and 200 µg/200µl by transferring respective amount of glucose from the standard glucose solution (1mg/ml) and adjusting it to a total volume of 200 µl by adding diluent buffer as mentioned in Table 2.
3. Add 0.5 ml of DNSA reagent to all the six test tubes. Mix well.
4. Keep in boiling water bath for 15 minutes.
5. Add 0.5 ml of 40 % Potassium sodium tartrate (Rochell's Salt) solution and mix it well.
6. Switch on the spectrophotometer and select the wavelength of 540 nm. First take the absorbance (OD) of Blank and make it zero.
7. Take the OD of all the tubes (No. 1-5). Wash the cuvettes each time after taking OD.

Table 2:

Tube No.	**Blank**	**1**	**2**	**3**	**4**	**5**
Conc. of Glucose (µg)	0.0	40	80	120	160	200
Vol. of Glucose std. taken (µl)	0.0	40	80	120	160	200
Vol. of diluent buffer added (µl)	200	160	120	80	40	0.0
Vol. of DNSA added (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Keep in Boiling Water Bath for 15 minutes						
Vol. of 40 % Rochell's Salt added	0.5	0.5	0.5	0.5	0.5	0.5
Absorbance at 540 nm						

8. Plot a standard curve of absorbance at 540 nm on "Y" axis versus concentration of glucose in µg/200 µl on "X" axis.

9. Record the value "x" of Unknown (aliquots taken at regular interval of time) from graph corresponding to the optical density reading of the test sample**.**

Observation and Result:

1. Check for the formation of proper beads. Beads should be hard and spherical as shown in Figure 3.

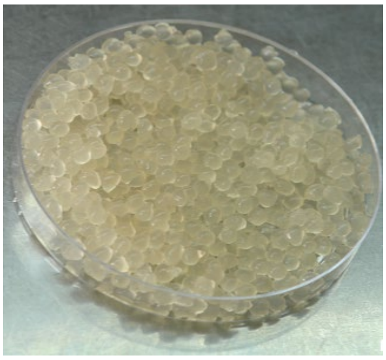
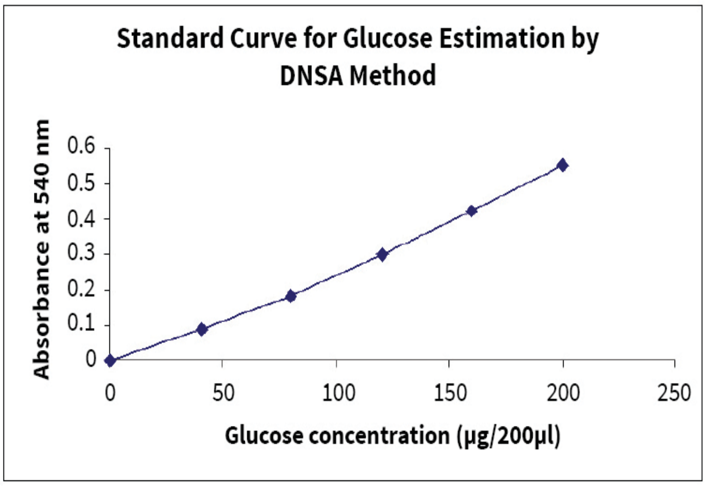


Fig 3: Yeast cells immobilized in alginate beads

2. Prepare the standard curve as shown below.



The absorbance at 540 nm increases with increase in glucose concentration

3. Note down the absorbance of the tubes I – V as shown in Table 3 and estimate the concentration of glucose at each time points.

Table 3: Absorbance of the aliquots at 540 nm at regular interval of time

Time (in minutes)	Absorbance at 540 nm
0	
30	
60	
90	
120	

Glucose concentration can be calculated by using the following formula:

$$\text{Glucose concentration in aliquots} = \frac{\text{Concentration of aliquot in "µg"}}{\text{Volume of aliquot in "µl"}}$$

= ………… x 5 µg/ml

Where, volume of the aliquot is 200 µl

Interpretation:

When the yeast cell suspension (mixed with a solution of Sodium alginate) is dripped into a solution containing multivalent cations (usually Ca^{2+}) the droplets form spheres as they fall, entrapping the cells in three-dimensional lattice of ionically cross-linked alginate.

The enzymatic activity of immobilized yeast cells is measured by the conversion of sucrose to glucose which in turn is detected by the DNSA method. During the invertase assay, concentration of glucose increases with time which proves that the biological activity is retained in the immobilized yeast cells.

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1	Beads are not formed properly	Calcium chloride solution was not kept on ice	Always place the Calcium chloride solution on ice
2	Beads are not spherical in shape	Cell suspension was added slowly	Cell suspension should be added very quickly
3	Sodium alginate solution is not homogenous	Sodium alginate is not dissolved properly	Sodium alginate solution should be heated while preparing
4	Standard and test samples give lower OD values than expected although the Blank is OK	Procedure was not carried out properly	Follow the entire procedure carefully
		Absorbance was not measured at correct wavelength	Measure absorbance at correct wavelength as mentioned in the brochure

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com

Symbol:

	Manufacturer		Do not use if package is damaged
LOT	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)	REF	Catalogue number

Identification No.: PIHTBC001
Rev No.: 07
Date of Issue: 2025-08