Lactase Assay Kit

Catalog No.: abx295091

Size: 96 tests

Detection Range: 12.5 U/ml – 2000 U/ml

Sensitivity: 3.94 U/ml

Storage: Store all components at 2-8°C. Store the Phenol Solution and Enzyme Solution in the dark.

Application: For detection and quantification of Lactase activity in animal tissue homogenates.

Introduction

Lactase is a key digestive enzyme which assists in the breakdown of lactose. Lactose is a disaccharide comprised of glucose and galactose joined by a β-1,4 glycosidic bond. When lactase catalyzes the hydrolysis of the β-1,4 glycosidic bond, glucose and galactose are released, allowing them to be absorbed by the intestine. The enzyme glucose oxidase catalyzes the breakdown of glucose, which releases hydrogen peroxide.

Abbexa's Lactase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lactase activity. In the presence of certain chromogens, the enzyme peroxidase catalyzes the breakdown of hydrogen peroxide to produce compounds with an absorbance maximum at 505 nm. The intensity of the color is proportional to the Lactase activity, which can then be calculated.

Kit components

1. 96-well microplate
2. Substrate: 1 vial
3. Buffer Solution: 10 ml
4. Stop Solution: 6 ml
5. Phenol Solution: 12 ml
6. Enzyme Solution: 12 ml
7. 50 mmol/L Glucose Standard Solution: 1.5 ml
8. Plate sealer: 2

Materials required but not provided

1. Microplate reader (495-510 nm, optimum wavelength 505 nm)
2. Micropipette
3. Centrifuge
4. Vortex mixer
5. Incubator
6. Double-distilled water
7. Normal saline (0.9% NaCl)
8. PBS (0.01 M, pH 7.4)
9. Mechanical homogenizer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue, and wash in cold PBS (0.01 M, pH 7.4). For each 20 mg of homogenate, add 180 µl of normal saline (0.9% NaCl). Homogenize by hand, using a mechanical homogenizer. Centrifuge the homogenate at 10,000 × g for 10 minutes. Collect the supernatant and assay immediately, or aliquot and store at -80°C for up to 1 month.

  Note: To calculate Lactase activity in tissue homogenates using the formula in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately **(abx097193)**.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Rat Ileal tissue homogenate	1
10% Rat jejunum tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- The incubation time and temperature at 37°C must be accurately maintained.
- Pipette samples gently up and down to mix before adding to wells. Avoid foaming or bubbles.

2. Reagents

- **Substrate Working Solution:** Dissolve one vial of Substrate with 8 ml of Buffer Solution, mix thoroughly. Store at 2-8°C for up to 1 month in the dark.
- **Chromogenic Reagent Working Solution:** For each well, prepare 200 µl of Chromogenic Reagent Working Solution by mixing 100 µl of phenol solution and 100 µl of enzyme solution. The Chromogenic Reagent Working Solution should be prepared immediately before use.
- **Standards:** Label 7 tubes with 40 mmol/L, 30 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, and 2 mmol/L. Add 80 µl, 60 µl, 40 µl, 30 µl, 20 µl, 10 µl, and 4 µl of 50 mmol/L Glucose Standard Solution to the 40 mmol/L, 30 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, and 2 mmol/L tubes respectively, followed by 20 µl, 40 µl, 60 µl, 70 µl, 80 µl, 90 µl, and 96 µl of double-distilled water, to prepare Standard Dilutions with concentrations 40 mmol/L, 30 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L, and 2 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	40	30	20	15	10	5	2
50 mmol/L Glucose Standard Solution (µl)	80	60	40	30	20	10	4
Double-distilled water (µl)	20	40	60	70	80	90	96

For the blank, or 0 mmol/L standard, use pure double-distilled water. The volume of each standard will be 100 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard, sample, and control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 25 µl of each Standard Dilution to the corresponding standard tubes.
3. Add 25 µl of sample to the corresponding sample tubes.
4. Add 50 µl of Substrate Working Solution to all tubes.
5. Mix thoroughly and incubate at 37°C for 20 minutes.
6. Add 25 µl of Stop Solution to all tubes.
7. Add 25 µl of sample to the corresponding control tubes.
8. Mix thoroughly and centrifuge at 1780 x g for 10 minutes.

9. Assign and record microplate well positions for each standard, sample, and control.
10. Add 8 µl of the supernatant to the corresponding microplate wells.
11. Add 200 µl of Chromogenic Reagent Working Solution to all wells.
12. Mix thoroughly for 10 seconds by tapping the plate or using an orbital shaker.
13. Incubate the plate at 37°C for 15 minutes.
14. Measure the OD of each well with a microplate reader at 505 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the Lactase activity in each sample well can be derived with the following formula:

1. Tissue samples:

Lactase activity in tissue samples can be calculated according to total protein concentration (which must be assayed separately).

Total Protein

One unit of Lactase activity is defined as the amount of 1 mmol of lactose hydrolyzed by 1 mg of tissue protein per minute at 37°C.

$$\text{Lactase activity (U/mg Protein)} = \frac{(\Delta A - b) \times 1000^{**} \times f}{a \times C_{Protein} \times 20^{*}}$$

where:

$C_{Protein}$	The concentration of protein in sample (mg protein/ml)
1000^{**}	1 µmol = 1000 nmol
20^{*}	Time of the enzymatic reaction (20 minutes)
ΔA	OD_{Sample} - $OD_{Control}$
f	The dilution factor of sample before testing

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.