Lactase Assay Kit

Catalog No.: abx295092

Size: 50 tests

Detection range: 2.0 U/ml – 1600 U/ml

Sensitivity: 2.0 U/ml

Storage: Store the detection reagent in the dark at 2-8°C. Store the rest of the kit components at 2-8°C.

Application: For detection and quantification of lactase activity in Human tissue homogenates and other biological fluids.

Introduction

Lactase in an enzyme located in the border of the small intestine of humans and mammals. Lactase allows organisms to digest whole milk by breaking down the lactose found in milk. Without lactase, lactose would not be broken down. Lactase is part of the β-galactosidase family of enzymes and is a glycoside hydrolase involved in the hydrolysis of lactose into glucose and galactose. In humans, lactase is coded by the LCT gene. Lactase will react with a substrate to produce a monosaccharide and hydrogen peroxide. Hydrogen peroxide and a detection reagent combined will produce a red product.

Abbexa's Lactase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lactase activity. The product has an absorbance maxima at 505 nm. The intensity of the color is proportional to the Lactase activity, which can then be calculated.

Kit components

1. Substrate: 1 vial
2. Diluent: 10 ml
3. Stop Solution: 5 ml
4. Detection Reagent: 50 ml
5. Glucose Standard Solution (5.5 mmol/L): 0.1 ml

Materials Required But Not Provided

1. Spectrophotometer (505 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Glucose Standard Solution (1.85 mmol/L):** Dilute Glucose Standard Solution (5.55 mmol/L) with double distilled water at a 1:2 ratio. Prepare before use.

2. 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:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 3500 x g for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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.

B. Assay Procedure

1. Label a sample tube and a control tube.
2. Add 25 µl of sample to the sample tube.
3. Add 50 µl of substrate to the sample tube and the control tube.
4. Mix the sample tube and control tube fully and incubate for 20 minutes at 37°C.
5. Add 25 µl of stop solution to the sample tube and control tube.
6. Add 25 µl of sample to the control tube.
7. Mix the sample tube and control tube fully and centrifuge at 4000 rpm for 10 minutes.
8. Take the supernatant and store on ice.
9. Label a blank, standard, sample and control tube.
10. Add 40 µl of double distilled water to the blank well.
11. Add 40 µl of Glucose Standard Solution (1.85 mmol/L) to the sample well.
12. Add 40 µl of supernatant to the sample and control well.
13. Add 1000 µl of detection reagent to the blank, standard, sample and control well.
14. Mix the blank, standard, sample and control well fully and incubate at 37°C for 15 minutes.

15. Set the spectrophotometer to 0 with double distilled water.
16. Measure the OD values of each well at 505 nm (using 1cm optical path cuvette).

C. Calculation of Results

1. Tissue homogenate samples:

One unit is defined as the amount of lactase in 1 mg of tissue that hydrolyzes 1 nmol of lactase per minute at 37°C and a pH of 6.

$$\text{Lactase activity (U/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times \frac{V_1}{t \times (C_{pr} \times V_2)} \times C \times 10^6$$

where:

ΔA_1	OD_{Sample} - $OD_{Control}$
ΔA_2	$OD_{Standard}$ - OD_{Blank}
V_1	The total volume of enzymatic reaction (0.1×10^{-3} L)
V_2	The volume of the sample (0.025 ml)
t	The enzymatic reaction time (20 minutes)
C_{pr}	Concentration of the protein in the sample (mgprot/ml)
C	The concentration of standard (1.85 mmol/L)
10^6	1 mmol/L = 10^6 nmol/L