Alpha-Galactosidase Assay Kit

Catalog No.: abx298864

Size: 100 Assays

Storage: Store Substrate and Positive Control at -20°C. Store all other kit components at 4°C.

Application: For quantitative detection of Alpha-Galactosidase (α-GAL) activity in tissue homogenates, cell lysates, cell culture supernatants, and other biological fluids.

Detection Range: 10 µmol/L – 1000 µmol/L

Introduction: Alpha-Galactosidase is a glycoside hydrolase found throughout the human body. It catalyzes the hydrolysis of terminal α-D-galactose moieties from glycolipids and glycoproteins.

Alpha-Galactosidase catalyzes the hydrolysis of glucoside, producing p-nitrophenol. The concentration of this reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 405 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 5 ml
4. Dye Reagent: 15 ml
5. Standard (1000 µmol/L): 1 ml
6. Substrate: 1 vial
7. Positive Control: 1 vial
8. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (405 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 2 ml of Reaction Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.
- **Positive Control Solution**
  Add 1 ml of distilled water to the Positive Control vial and mix thoroughly. Ensure that the Positive Control has completely dissolved. Aliquot 0.01 ml of this solution into 0.99 ml distilled water to prepare the Positive Control Solution.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 10,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

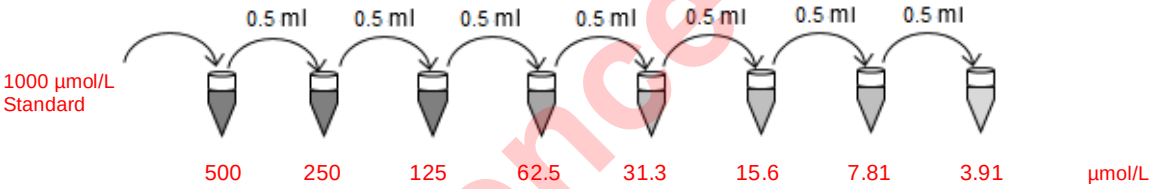
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 10,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 8 tubes with 500 µmol//L, 250 µmol//L, 125 µmol//L, 62.5 µmol//L, 31.3 µmol//L, 15.6 µmol//L, 7.81 µmol//L and 3.91 µmol//L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1000 µmol//L Standard to the 1st tube (500 µmol//L) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Label the sample, control, positive control, standard, and blank wells on the 96 well microplate. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to the sample wells.
4. Add 10 µl of distilled water to the control wells.
5. Add 10 µl of Positive Control Solution to the positive control wells.
6. Add 20 µl of Substrate Solution to the sample, control, and positive control wells.
7. Add 20 µl of Reaction Buffer to the sample, control, and positive control wells. Mix thoroughly. At this stage, the standard wells should be empty, with no liquid added.
8. Incubate at 37°C for 30 minutes.
9. Add 50 µl of prepared standards to the standard wells.
10. Add 200 µl of Dye Reagent to the blank wells, then add 150 µl of Dye Reagent to all other wells. The total volume in all wells should now be 200 µl.
11. Tap the plate gently to mix.
12. Read and record absorbance at 405 nm.

C. Calculations

One unit of Alpha-Galactosidase activity is defined as the amount of enzyme required to produce 1 µmol of p-nitrophenol per hour.

Alpha-Galactosidase activity per mg of protein:

$$\alpha\text{GAL}\ (\text{U/mg}) = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}}{\text{C}_{\text{Protein}} \times \text{V}_{\text{Sample}} \times \text{T}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} = \frac{10}{\text{C}_{\text{Protein}}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}}$$

Alpha-Galactosidase activity per g of sample:

$$\alpha\text{GAL}\ (\text{U/g}) = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times \text{V}_{\text{Assay}}}{\text{W} \times \text{V}_{\text{Sample}} \times \text{T}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} = \frac{10}{\text{W}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}}$$

Alpha-Galactosidase activity per 10^4 cells or bacteria:

$$\alpha\text{GAL}\ (\text{U}/10^4\ \text{cells}) = \frac{\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}} \times \text{V}_{\text{Assay}}}{\text{N} \times \text{V}_{\text{Sample}} \times \text{T}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}} = \frac{10}{\text{N}} \times \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}}{\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}}$$

where:

$\text{C}_{\text{Protein}}$	Concentration of protein (in mg/ml)
$\text{C}_{\text{Standard}}$	Concentration of highest standard (1 mmol/L = 1000 µmol/L = 1 µmol/ml)
T	Reaction time (0.5 hours)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Volume of assay buffer (1 ml)
V_{Sample}	Volume of sample (0.01 ml)
$\text{V}_{\text{Standard}}$	Volume of standard (0.05 ml)