CREATIVE BIOMART®
Assay Kit

Alpha-Galactosidase Assay Kit

Product Information

Cat.No.

Kit-0869

Product Overview

The assay is initiated with the enzymatic hydrolysis of the glucoside by a-Galactosidase. The enzyme catalysed reaction products p-nitrophenol, can be measured at a colorimetric readout at 400 nm.

Description

Alpha-galactosidase is a glycoside hydrolase enzyme that hydrolyses the terminal alpha-galactosyl moieties from glycolipids and glycoproteins. It is encoded by the GLA gene. Two recombinant forms of alpha-galactosidase are called agalsidase alfa (INN) and agalsidase beta (INN). This enzyme is a homodimeric glycoprotein that hydrolyses the terminal alpha-galactosyl moieties from glycolipids and glycoproteins. It predominantly hydrolyzes ceramide trihexoside, and it can catalyze the hydrolysis of melibiose into galactose and glucose.

Storage

Shipped and store at 4 °C for 6 months.

Synonyms

Alpha-Galactosidase Kit; Alpha-Galactosidase Assay; Alpha-Galactosidase; EC 3.2.1.22; Melibiase; GLA; GALA; 9025-35-8

Size

100 Assays

Kit Components

96-Well Microplate: 1 plate
Assay Buffer: 100 ml x 1; 4 °C
Reaction Buffer: 4 ml x 1; 4 °C
Substrate: Powder x 1; -20 °C
Stop Solution: 15 ml x 1; 4 °C

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Standard (5μmol/mL p-nitrophenol solution): 1 ml x 1; 4 °C
Note:
Substrate: For each tube, add 2.5 ml distilled water to dissolve before use.

Materials Required but Not Supplied

1. Microplate reader to read absorbance at 400 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice
9. Water bath

Target Species

Detection and Quantification of Alpha-Galactosidase (a-GAL) Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

Preparation

1. For cell and bacteria samples
Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 15,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
2. For tissue samples
Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 15,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.
3. For Standard solution
Dilute the standard solution to 200, 100, 50, 25, 12.5, 6.25, 0 nmol/mL with Reaction Buffer.

Assay Protocol

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1. Preheat the plate reader for more than 30 minutes, adjust the wavelength to 400 nm, and adjust to zero with distilled water.
2. Sample determination, (add the following reagents in sequence to the 96-well plate):

Reagent	Sample	Control	Standard
Substrate	25ul	-	-
Distilled water	-	25ul	-
Reaction Buffer	35ul	35ul	-
Sample	10ul	10ul	-
Mix quickly and keep at 37 ° C for 30 min			
Standard	-	-	70ul
Stop Solution	130ul	130ul	130ul

Mix well, measure absorbance A at 400 nm, and calculate ΔA=A sample - A control.

Analysis

The standard curve was established based on the absorbance of the standard tube (x: absorbance of each standard tube minus the absorbance of a standard tube with a concentration of zero) and concentration (y, nmol/mL). The ΔA was brought into the standard curve to calculate the product concentration (nmol/mL) produced by the sample.

(1) Calculated by sample protein concentration:

Unit Definition: One unit is defined as the enzyme required to produce 1 nmol of p-nitrophenol per mg of tissue protein per hour.

a-GAL activity (nmol/h /mg prot)=(y×V total)÷(V sample×Cpr)÷T=14×y÷Cpr

(2) Calculated by sample weight:

Unit Definition: One unit is defined as the enzyme required to produce 1 nmol of p-nitrophenol per hour per g tissue.

a-GAL activity (nmol/h /g fresh weight) = (y × V total) ÷ (W × V sample ÷ V assay) ÷ T = 14 × y ÷ W

(3) Calculated by the number of bacteria or cells:

Unit Definition: One unit is defined as the enzyme required to produce 1 nmol of p-nitrophenol per hour per 10,000 bacteria or cells.

a-GAL activity (nmol/h /10^4 cell) = (y × V total) ÷ (500 × V sample ÷ V assay) ÷ T = 0.028 × y

Cpr: sample protein concentration, mg/mL;

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V total: the total volume of the reaction system, 0.07mL;
V sample: the volume of sample, 0.01 mL;
V assay: the volume of Assay buffer, 1 mL;
W: the weight of sample, g;
500: total number of cells or bacteria, 5 million;
T: the reaction time, 0.5 h.

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