SunLongBiotechCo.,LTD
Tel:0086-571-56623320Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

α-L-Rhamnosidase Activity Assay Kit (acidic conditions)

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer

Catalog Number: AK0707-50T-24S

Size:50T/24S

Product composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle, and if you have any questions, please contact the staff of Sunlong in time.

Reagent	Size	Preservation Condition
Reagent I	Liquid 60 mL × 1	2-8 °C
Reagent II	powder × 1	-20 °C
Reagent III	Liquid 55 mL × 1	2-8 °C
Standard	Liquid 1 mL × 1	2-8 °C

Solution Preparation:

1. **Reagent II:** Add 26.7mL of Reagent I to fully dissolve before use, which can be ultrasonic to promote dissolution. The dissolved reagent can be stored at -20°C for 4 weeks after aliquoting. Avoid repeated freezing and thawing;
2. **Standard:** 5 μmol/mL p-nitrophenol solution. Add 700 μL distilled water to 100 μL of 5 μmol/mL p-nitrophenol solution to prepare 0.625 μmol/mL p-nitrophenol solution, which is used as a standard for the standard tube in the operation table.

Product Description:

α-L-Rhamnosidase (EC 3.2.1.40) facilitates the hydrolysis of terminal α-L-rhamnose residues from flavonoid glycosides and terpenes, including rutin, hesperidin, and naringin. The optimal pH for the majority of α-L-rhamnosidases typically falls within the acidic to near-neutral range. Specifically, fungal α-L-rhamnosidases generally exhibit a preference for more acidic conditions, with an optimal pH range of 4.0–6.5. In contrast, bacterial-derived α-L-rhamnosidases tend to favor near-neutral to alkaline environments, having an optimal pH range of 5.0–8.0. **This particular kit is designed for the assessment of α-L-rhamnosidase activity under acidic conditions.**

In acidic settings, α-L-rhamnosidase catalyzes the reaction with p-nitrophenol-α-rhamnoside (PNPR), yielding yellow p-nitrophenol (PNP), which displays a maximum absorption peak at 400 nm. By quantifying the rate at which PNP accumulates at this wavelength, one can determine the extent of acidic α-L-rhamnosidase activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, benchtop centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, mortar/homogenizer/cell ultrasonic disruptor, ice, and distilled water.

Operation procedure:

I. Sample processing: (the sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. Tissue: According to the ratio of tissue mass (g): volume of reagent (mL) is 1:5~10 (it is recommended to weigh about 0.1g of tissue and add 1mL of reagent I), and homogenize in ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ and take the supernatant on ice before test.
2. Bacteria or cells: Collect bacteria or cells into a centrifuge tube first, and discard the supernatant after centrifugation. According to the number of bacteria or cells (10^6): the volume of reagent (mL) is 5~10:1 ratio (it is recommended that adding 1 mL Reagent I into 5 million of bacteria or cells). Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 10 seconds, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃ and take the supernatant on ice before test.

II. Determination procedure:

1. Preheat the spectrophotometer reader for 30 minutes, adjust wavelength to to 400nm, set spectrophotometer counter to zero with distilled water.
2. Operation table (add the following reagents in a 2 mL EP tube)

Reagent Name (μL)	Test tube (T)	Contrast tube (C)	Standard tube(S)	Black tube (B)
Reagent II	425	-	-	-
sample	50	50	-	-
Mix well and react at 40°C for 30min			-	-
Standards	-	-	50	-
distilled water	-	-	-	50
Reagent I	-	-	425	425
Reagent III	880	880	880	880
Reagent II	-	425	-	-

Dissolve the sediment fully and stand at room temperature for 2 min, measure the absorbance value at 400nm, calculate $\Delta A = A_T - A_C$ and $\Delta A_S = A_S - A_B$. A control tube is required for each assay tube. Blank and standard tubes only need to be measured 1-2 times.

III. Calculate the activity of α-L-rhamnosidase

(1) Protein concentration

Definition of units: 1 μmol of p-nitrophenol per minute per mg of tissue protein is defined as one enzymatic active unit.

$$\text{α-L-rhamnosidase activity (U/mg prot)} = \Delta A \times C_S \div \Delta A_S \times V_S \div (V_S \times Cpr) \div T \times F$$
$$= 0.0208 \times \Delta A \div \Delta A_S \div Cpr \times F$$

(2) Sample mass

Definition of units: The production of 1 μmol p-nitrophenol per minute per g of tissue is defined as one enzymatic living unit.

α-L-Rhamnosidase activity (U/g mass) = $\Delta A \times C_S \div \Delta A_S \times V_S \div (W \times V_S \div V_T) \div T \times F$

$$= 0.0208 \times \Delta A \div \Delta A_S \div W \times F$$

(3) Germ or cells number

Definition of units: 1 μmol of p-nitrophenol per minute produced per 1.06 million bacteria or cells is defined as one enzymatic active unit.

α-L-rhamnosidase activity (U/10^6 cell) = $\Delta A \times C_S \div \Delta A_S \times V_S \div (N \times V_S \div V_T) \div T \times F$

$$= 0.0208 \times \Delta A \div \Delta A_S \div N \times F$$

C_S: p-nitrophenol solution concentration, 0.625μmol/mL; Cpr: sample protein concentration, mg/mL; V_S: sample volume added to the reaction system, 0.05mL; V_T: avolume of reagent I, 1mL; W: sample mass, g; N: total number of cells or bacteria, in 10^6; T: reaction time, 30min; F: dilution factor.

Note:

1、Should the measured absorbance value exceed 1.2 or the change in absorbance (ΔA) surpass 1, it is advisable to dilute the sample using Reagent I or reduce the reaction duration at 40°C. Conversely, if the absorbance value approaches that of the blank or if ΔA is excessively low, increasing the sample volume or prolonging the reaction time at 40°C would be appropriate. It is crucial to adjust the calculation formula accordingly during the final computation.

Experiment example:

1、Weigh the mold on 0.1020g of oranges, add reagent I for ice bath homogenization, operate according to the determination steps, and calculate $\Delta A = A_T - A_C = 0.368-0.043 = 0.325$, $\Delta A_S = A_S - A_B = 0.379-0 = 0.379$, bring in the formula to calculate:

α-L-rhamnosidase activity (U/g mass) = $0.0208 \times \Delta A \div \Delta A_S \div W \times F = 0.1749$ U/g mass