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Wako

Code No. 119-01021 (1g)

Kitalase
キタラーゼ

Kitalase is a lytic enzyme which principally shows β-1,3-glucanase activity. It is reported that Kitalase also has protease, hemicellulase, pectinase and amylase activity. It is used to lyse cell walls and cell membranes for the preparation of protoplasts from fungi.

Source : *Rhizoctonia solani*
Appearance : Pale brown ～ brown, powder
Endo-β-1,3-glucanase activity : Indicated on the label.

Assay Method of Endo-β-1,3-glucanase activity
1. Reagents

A. 50 g/L Phenol solution
B. 0.5 mol/L HCl
C. 0.1 mol/L Citric acid solution
D. 0.2 mol/L Disodium hydrogen phosphate solution
E. McIlvain buffer (pH 5.0)
Add Reagent D gently to 97 mL of Reagent C until pH 5.0.
F. Dilution solution
Dilute Reagent E 23.8-fold by adding water.
G. Substrate solution
Measure 2 g of dried Curdlan (β-1,3-glucan) exactly and add 100 mL of water. Stir the solution well to get homogeneous suspending solution.
Prepare this solution before using.
H. Enzyme solution
Measure 50 mg of Kitalase exactly and add dissolve it in Reagent F for a volume of 200 mL. Dilute 10 mL of the solution with Reagent F to bring the volume up to 100 mL.
[Note]
When the absorbance after enzyme reaction is over 0.3, dilute the Reagent H using Reagent F to the absorbance between 0.2 ～ 0.3.
I. 50 μg/mL Glucose standard solution
Measure 500 mg of glucose exactly and dissolve it in water for a volume of 100 mL. Dilute 100 μL of the solution with 900 μL of water.
And dilute 100 μL of the solution with 900 μL of water.

2. Procedure

2-1. Assay

Reagent	Test	Blank
H	5.0 mL	5.0 mL
F	10.0 mL	10.0 mL

Reagent	Test	Blank
	Mix the solution with a stirrer at 37℃ for 5 minutes. Trans* 37℃で5分間、スターラーで撹拌する。	
G	5.0 mL	–
	Mix the solution with a stirrer at 37℃ for 30 minutes. Trans* 37℃で30分間、スターラーで撹拌する。	–
B	5.0 mL	5.0 mL
G	–	5.0 mL
	Filter thought 0.45 μm filter. Trans* 0.45 μmのフィルターでろ過する。	
Filtered solution	0.5 mL	0.5 mL
A	0.5 mL	0.5 mL
Sulfuric acid	2.5 mL	2.5 mL
	Add immediately and shake vigorously. Let stand for 30 minutes. Record the absorbance at 490 nm with water as a reference using a 10 mm cell. Trans* 速やかに加え、激しくかき混ぜ、30分間放置する。吸収セル10mmを用い、波長490nmにおける吸光度を水を対照液として測定する。	

2-2. Standard

Reagent	Standard	Blank
I	0.5 mL	–
Water	–	0.5 mL
A	0.5 mL	0.5 mL
Sulfuric acid	2.5 mL	2.5 mL
	Add immediately and shake vigorously. Let stand for 30 minutes. Record the absorbance at 490 nm with water as a reference using a 10 mm cell. Trans* 速やかに加え、激しくかき混ぜ、30分間放置する。吸収セル10mmを用い、波長490nmにおける吸光度を水を対照液として測定する。	

3. Unit Definition

One unit is the amount of enzyme which liberates soluble sugar equivalent to 1 μmol of glucose per minute under above conditions.

(Calculation)

$$\text{Units/g} = \frac{(E_1\text{-}E_0) \times \frac{S_2}{10} \times 25}{(E_{STD}\text{-}E_{Bl}) \times 180} \times \frac{1}{30} \times \frac{1}{S_1 \times \frac{5}{\text{dilution rate}}}$$

E_1 : Absorbance of test
E_o : Absorbance of blank
E_{STD} : Absorbance of standard
E_{Bl} : Absorbance of standard's blank
S_1 : The measured volume of Kitalase (g)
S_2 : The measured volume of glucose (mg)

Trans*

3. 単位の定義

上記の条件下で、1 分間に 1 μmol のグルコースに相当する可溶性糖を遊離する酵素量を 1 unit とする。

(計　算)

$$\text{Units/g} = \frac{(E_1\text{-}E_0) \times \frac{S_2}{10} \times 25}{(E_{STD}\text{-}E_{Bl}) \times 180} \times \frac{1}{30} \times \frac{1}{S_1 \times \frac{5}{\text{希釈倍率}}}$$

E_1 : 本試験の吸光度
E_o : 空試験の吸光度
E_{STD} : 標準品の吸光度
E_{Bl} : 標準品空試験の吸光度
S_1 : 量り取った本品の質量 (g)
S_2 : 量り取ったグルコースの質量 (mg)

【Note】

Depending on the buffer, clogging might occur when filtering the Kitalase solution. It does not affect the activity. Please dispense the solution and filter them respectively.

Trans*

溶解するバッファーによっては、溶解後ろ過滅菌して使用する際に、目詰まりを生じる場合がございますが、活性に影響はございません。数回に分けてろ過滅菌を行って下さい。

【Storage】 Store at 2 ～ 10℃.

【Package】 1 g

＊ : Trans is the Japanese translation.

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