Yatalase

Code No. T017 Size: 2 g

${\bf Example\ of\ protoplast\ preparation:}$

(1) Aspergillus oryzae

Medium Czapek-Dox + 0.5% Casamino acid (pH 5.6) Culture 30°C, 20 hr, Shake culture (Rotary at 140 rpm)

Conditions 2% Yatalase solution 0.6 M (NH4)₂SO₄

50 mM Maleate Buffer (pH 5.5)

30°C Reciprocal shaking (at 60 - 70 rpm) 2 - 3 hr

(2) Aspergillus kawachi

Medium Same as in (1)

Culture 30°C, 20 hr, Stationary culture

Conditions Same as in (1)

(3) Aspergillus terreus, Penicillium citrinum, Penicillium lanosum,

Tricoderma koningii

Medium Dextrin-peptone (pH 5.5)

Culture Same as in (1) Conditions Same as in (1)

(4) Mucor hiemalis, Rhizopus nigricans
Medium 2% Malt extract

Culture 30° C, 12 hr, Stationary culture

(Sporangiospores in germ)

Conditions 2% Yatalase solution

0.5 M MgSO₄

50 mM Maleate Buffer (pH 5.5)

30°C Reciprocal shaking (at 60 - 70 rpm) 4 hr

(5) Pleurotus ostreatus, Coprinus cinereus, Lentinus edodes

 $\begin{array}{ll} \text{Medium} & \text{OSG or MYG medium (pH 5.5)} \\ \text{Culture} & 25-30^{\circ}\text{C}, 3-4 \text{ days, Stationary culture} \\ \end{array}$

Conditions 2% Yatalase solution

0.6 M MgSO₄

50 mM Maleate Buffer (pH 5.5)

30°C Reciprocal shaking (at 60 - 70 rpm) 2 - 3 hr

(6) Monascus sp.

Medium Same as in (3)

Culture 25° C, 20 hr, Shake culture (Rotary at 140 rpm)

Conditions Same as in (1)

Description:

This product was prepared from lipuid culture supernatant of *Corynebacterium* sp. OZ-21. This product has complex lytic activies of fungal cell mainly consisting of chtinase and chitobiase activity.

Origin: *Corynebacterium* sp. OZ-21

Appearance: Lyophilized powder (containing lactose as the excipient)

Storage: 4°C, dry condition

Unit definition:

[Chitinase activity]

1 unit is defined as the amount of enzyme required to release 1 μ mol of N-Acetylglucosamine from 0.5% chitin powder solution in 1 min at 37 °C, pH 6.0.

[Chitobiase activity]

1 unit is defined as the amount of enzyme required to release 1 μ mol of p-Nitrophenol from p-Nitropheny-N-acetyl- β -D-glucosaminide solution in 1 min at 37°C, pH 6.0.

[Lytic activity]

1 unit is defined as the amount of enzyme required to cause a 1% decrease in absorbance at 660 nm in 1 hour under the assay conditions described below.

Solutions

Solution A (Substrate solution);

Harvest a culture of Aspergillus oryzae (cultured in dextrin-peptone medium at 25 to 30° C for 1 to 2 days) and homogenize on a Waring blender (16,000 rpm, 3 min). Subsequently, apply a French press process to produce fragments. Then, wash the fragments throughly with water over filter paper , and then with acetone, followed by diethyl ether. Air-dry the pellet and suspend in 0.1 M acetate buffer, pH 6.0, to give 0.125% (w/v). Solution B (Enzyme solution);

Dissolve the enzyme in sterile purified water (0.8 mg/ml).

Assay system

- 1. Transfer 4 ml of solution A to a test tube.
- 2. Add 1 ml of solution B and immediately vortex.
- 3. Incubate at 37° C by gently shaking for 1 hr.
- 4. Read the absorbance at 660 nm.

Quality Control Data:

Please see the Certificate of Analysis (CoA) for each lot. You can download the CoA on Takara Bio website.

Manufactured by Ozeki corporation.

Note

This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc. Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from Takara Bio Inc.

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Yatalase

Code No. T017 容量: 2g

●プロトプラスト調製条件例

(1) Aspergillus oryzae

培地 Czapek-Dox + 0.5% Casamino acid (pH5.6) 培養条件 30℃, 20 hr., Shake culture (Rotary at 140 rpm)

調製条件 2% Yatalase solution 0.6 M (NH₄)₂SO₄

50 mM Malasta Puffor (nU5 5

50 mM Maleate Buffer(pH5.5)

30°C Reciprocal shaking (at 60 \sim 70 rpm) 2 \sim 3 hr.

(2) Aspergillus kawachi

培地 Same as in (1)

培養条件 30°C, 20 hr., Stationary culture

調製条件 Same as in (1)

(3) Aspergillus terreus, Penicillium citrinum, Penicillium lanosum, Tricoderma koningii

培地 Dextrin-peptone (pH5.5)

培養条件 Same as in (1) 調製条件 Same as in (1)

(4) Mucor hiemalis, Rhizopus nigricans

培地 2% Malt extract 培養条件 30℃,12 hr., Stationary culture

(Sporangiospores in germ)

調製条件 2% Yatalase solution

0.5 M MgSO₄

50 mM Maleate Buffer (pH5.5)

 30° C Reciprocal shaking (at $60 \sim 70 \text{ rpm}$) 4 hr.

(5) Pleurotus ostreatus, Coprinus cinereus, Lentinus edodes

培地 OSG or MYG medium (pH5.5)

培養 25 ~ 30℃, 3 ~ 4 days, Stationary culture

調製条件 2% Yatalase solution

0.6 M MgSO₄

50 mM Maleate Buffer (pH5.5)

30°C Reciprocal shaking (at $60 \sim 70 \text{ rpm}$) $2 \sim 3 \text{ hr}$.

(6) Monascus sp.

培地 Dextrin-peptone (pH5.5)

培養条件 25℃, 20 hr., Shake culture (Rotary at 140 rpm)

調製条件 Same as in (1)

●製品説明

本製品は、Corynebacterium sp. OZ-21 の培養上清より調製された、キチナーゼ、キトビアーゼを主体とする複合酵素剤である。

●由来 Corynebacterium sp. OZ-21

●形状 凍結乾燥粉末(賦形剤として乳糖を含む)

●保存 4°C、乾燥状態で保存

● 活性の定義

[キチナーゼ活性]

37℃、pH6.0 において 0.5 %キチン溶液から 1 分間に 1 µmol の *N*-Acetylglucosamine を遊離する酵素活性を 1U とする。

[キトビアーゼ活性]

37°C、pH6.0 において p-Nitropheny-N-acetyl- β-D-glucosaminide 溶液 から 1 分間に 1 μ mol の p-Nitrophenol を遊離する酵素活性を 1 U とする。

[細胞壁溶解活性]

下記の条件で 1 時間に 660 nm における濁度を 1%減少させる酵素活性を 1U とする。

測定試薬

_____ 試薬 A (基質液);

Aspergillus oryzae 菌体(Dextrin-Peptone 培地、 $25\sim30$ で $1\sim2$ 日間 振とう培養)をワーリングブレンダーでホモジナイズする(16,000 rpm, 3 分間)。次に菌糸体が小断片化するまでフレンチプレス処理を行い、ろ 紙上で充分に水洗いした後、アセトンさらにジエチルエーテルで洗浄する。これを風乾したもの(細胞壁画分)を 0.125%になるように 0.1 M 酢酸緩衝液(pH6.0)に均一に懸濁し、基質液とする。

試薬 B (酵素液);

0.8 mg/ml となるように滅菌精製水に溶解する。

測定手順

試験管にA液4mlをとる。

- B 液 1 ml を添加し、すばやく試験管ミキサーで良く混合して 37℃で 反応を開始する。
- 3. 1時間おだやかに振とう後、660 nm の濁度をすばやく測定する。

● 品質管理

性能試験結果については、各ロットの Certificate of Analysis (CoA) をご覧ください。CoA はタカラバイオウェブサイトからダウンロードできます。

●製造元 大関株式会社

● 注意

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