

FUJIFILM

Wako

Code No. 070-05661 (1,000 units)

## Glutamate Oxaloacetate Transaminase, from Porcine Heart グルタミン酸オキサロ酢酸トランスアミナーゼ, ブタ心臓由来

This product is for laboratory research use only ; use in any such application is the responsibility of the user.

**Appearance** : Lyophilized powder from the solution of 0.05 mol/L Phosphate Buffer, pH 7.0

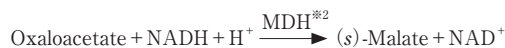
**Source** : Porcine heart

**Activity** : Indicated on the label

**CAS No.** : 9000-97-9

### Assay Method

#### 1. Principle



\*1 GOT : Glutamate Oxaloacetate Transaminase

\*2 MDH : Malate Dehydrogenase

#### 2. Reagents

- 0.1 mol/L Phosphate Buffer, pH 7.8
- 0.005 mol/L Phosphate Buffer, pH 7.8 (Store at 5°C)
- 2 mol/L Potassium Hydroxide
- 4 mol/L Sodium Hydroxide
- 0.3 mol/L L-Aspartate Solution  
Dissolve 4.0 g of L-Aspartate in 50 mL of Reagent A. Adjust to pH 7.8 with Reagent C and add Reagent A to bring the final volume up to 100 mL.
- 0.62 mol/L 2-Oxoglutarate Solution  
Dissolve 4.5 g of 2-Oxoglutarate in 20 mL of water. Adjust to pH 6 to 7 with Reagent D and add water to bring the final volume up to 50 mL.
- 0.008 mol/L NADH Solution  
Dissolve 57 mg of  $\beta$ -Diphosphopyridine Nucleotide Disodium (reduced form) in cold Reagent B to bring the final volume up to 100 mL. Store at 5°C.

#### H. MDH Solution

Dissolve MDH in Reagent A to bring the final activity up to 160 units/mL. Store at 5°C.

#### I. Enzyme Solution

Dissolve this product in Reagent A to bring the final activity up to 0.1 to 0.4 units/mL.

Trans \*

#### 2. 試薬

- 0.1mol/L リン酸緩衝液、pH 7.8
- 0.005mol/L リン酸緩衝液、pH 7.8 (5℃に保つ)
- 2mol/L 水酸化カリウム溶液
- 4mol/L 水酸化ナトリウム溶液
- 0.3mol/L L- アスパラギン酸溶液  
L- アスパラギン酸 4.0g を A 液 50mL に溶解し、C 液を用いて pH 7.8 に調整した後、A 液を加え 100mL にする。
- 0.62mol/L 2- オキシグルタル酸溶液  
2- オキシグルタル酸 4.5g を水 20mL に溶解し、D 液を用いて pH 6 ～ 7 に調整した後、水を加え 50mL にする。
- 0.008mol/L NADH 溶液  
 $\beta$  - ジホスホピリジンヌクレオチド二ナトリウム、還元型 57mg を氷冷した B 液に溶解し、10mL にする (5℃に保つ)。
- MDH 溶液  
りんご酸脱水素酵素を A 液に溶解し、160units/mL になるように調整する (5℃に保つ)。
- 酵素溶液  
本品を A 液に溶解し、0.1 ～ 0.4units/mL となるように調整する。

#### 3. Procedure

| Reagent                            | Test   | Blank  |
|------------------------------------|--------|--------|
| E                                  | 2.6 mL | 2.6 mL |
| F                                  | 0.1 mL | 0.1 mL |
| G                                  | 0.1 mL | 0.1 mL |
| H                                  | 0.1 mL | 0.1 mL |
| Pre-incubate at 37°C for 5 minutes |        |        |
| I                                  | 0.1 mL | —      |
| A                                  | —      | 0.1 mL |

Measure the absorbance at 340 nm wavelength at 2 and 7 minutes from the start of the measurement with water as the blank.

#### 4. Unit Definition

One GOT unit is the amount of enzyme which causes the transamination of 1  $\mu$ mol of L-Aspartate for 1 minute at 37°C and pH 7.8 with L-Aspartate and 2-Oxoglutarate as the substrate.

#### (Calculation)

$$A = (E_1 - E_0) \times \frac{1}{6.22} \times 3.0 \times \frac{1}{0.1 \times S} \times \frac{1}{5}$$

A : Activity (units/mg)

$E_1$  : Absorbance change per minute in Test

$E_0$  : Absorbance change per minute in Blank

3.0 : Total amount of reaction solution (mL)

0.1 : Amount of the assumption of diluted Reagent I (mL)

S : Amount of weighed this product for preparation of Reagent I (mg/mL)

6.22 : Milli-molar extinction coefficient of NADH in 340 nm

5 : Reaction time (min)

Trans \*

#### 4. 単位の定義

上記の条件で、L- アスパラギン酸と 2- オキシグルタル酸を基質として、pH 7.8、37°Cにおいて1分間に1  $\mu$ molのアスパラギン酸のアミノ基を転移させる酵素量を 1unit とする。

#### (計 算)

$$A = (E_1 - E_0) \times \frac{1}{6.22} \times 3.0 \times \frac{1}{0.1 \times S} \times \frac{1}{5}$$

A : 活性 (units/mg)

$E_1$  : 本試験の吸光度変化

$E_0$  : 空試験の吸光度変化

3.0 : 総液量 (mL)

0.1 : 希釈した本品溶液の使用量 (mL)

S : I 液で量りとした本品量 (mg/mL)

6.22 : 340nm における NADH のミリモル吸光係数

5 : 反応時間 (分)

**Storage** : Store at -20°C

**Package** : 1,000 units

\* : Trans is the Japanese translation.

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