

FUJIFILM**Wako**Code No. 147-08801 (5 g)
145-08802 (25 g)

Nattokinase

ナットウキナーゼ

Source : *Bacillus subtilis* var. *natto***Appearance** : White ~ slightly brown, crystalline powder ~ powder**Activity** : Indicated on the label.**Optimum pH** : 7.8**Optimum temperature** : 37 °C**Molecular weight** : approximately 27,724

[Assay Method]

1. Reagents

- (1) Reagent Solution A (5 mmol/L Substrate Solution) (prepare right before use)
Synthetic substrate (S-2222:Bz-Ile-Glu-(OR)-Gly-Arg-pNA)
7.4 mg corresponding amount⁽¹⁾ + add. Dimethylsulfoxide (DMSO) 2 mL.
- (2) Reagent Solution B [Borate Buffer (pH7.8)]
Boric Acid 5.0 g + Sodium Tetraborate Decahydrate 1.9 g + NaCl 1.2 g + H₂O 400 mL → adjust the solution to pH 7.8 with 0.1 mol/L NaOH or 0.1 mol/L HCl + H₂O (→500 mL).
- (3) Reagent Solution C (Standard Test Diluting Solution)
Reagent Solution B 270 mL + DMSO 30 mL.
- (4) Reagent Solution D (Standard Test Solution, concentration ; 50 μmol/L)
p-Nitroaniline 13.8 mg (correctly) → put it in a whole flask (volume 10 mL) + add. DMSO (→10 mL) → 1 mL is taken from a whole amount. (use the whole pipette) → put it in a whole flask (volume 100 mL) + add. Reagent Solution C (→100 mL) → 20 mL is taken from a whole amount. (use the whole pipette) + add. Reagent Solution C 20 mL.
- (5) Reagent Solution E (Test Solution) (prepare right before use)
Dilute *Nattokinase* with water to make 0.5~0.6 IU/mL.

2. Standard Test

Reagent Solution D (50 μmol/L) 3 mL → By the JIS K 0115 (General rules for molecular absorptiometric analysis), record the absorbance at 405 nm (25 °C) with water as a reference, and calculate the absorbance (mmol molecular extinction coefficient) of *p*-Nitroaniline (1 μmol/mL).

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3. Handling

	Main test	Blank test
Reagent Solution A	0.3 mL	0.3 mL
Reagent Solution B	2.4 mL	2.4 mL
Warm it for 5 min at 37 °C .		
Reagent Solution E	0.3 mL	—
Water	—	0.3 mL

Immediately, by the JIS K 0115 (General rules for molecular absorptiometric analysis), record the absorbance at 405 nm with water as a reference for 1 min.

4. Calculation

$$A = (\Delta E_1 - \Delta E_2) \times \frac{1}{B} \times 3.0 \times \frac{1}{S \times 0.3}$$

A : Activity (IU/g)

 ΔE_1 : change in absorbance of main test for 1 min. ΔE_2 : change in absorbance of blank test for 1 min.

S : Enzyme Concentration (g/mL)

B : mmol molecular extinction coefficient of *p*-Nitroaniline

3.0 : total liquid measure (mL)

0.3 : liquid measure of this test used (mL)

- (1) The substrates may contain additives, and thus convert before use.

5. Unit Definition

One unit is the amount of enzyme which liberate 1 μmol of *p*-Nitroaniline in minute at 37 °C .

[Storage] Store at 2~10 °C in the dark.**[Packages]**

Code No.	Package size
147-08801	5 g
145-08802	25 g

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