

Nattokinase Analytical Method

1. Add 100 $\mu\ell$ of test sample to 300 $\mu\ell$ of 0.1M Tris-HCl (pH 7.8) containing 10mM CaCl₂.
2. Prewarm the mixture in 30°C water bath for 5 min.
3. Add 300 $\mu\ell$ of 1.2% fibrin (0.12g fibrin is dissolved in 8ml of 0.2N NaOH and adjust to pH 7.8 with 6N HCl ; final volume is adjusted to 10ml with distilled water) to the pre-warmed mixture (except negative control).
4. Incubate the mixture at 30°C for 10 min.
5. To stop the enzyme reaction, add 600 $\mu\ell$ of 0.11M trichloroacetic acid containing 0.22M sodium acetate and 0.33M acetic acid to the mixture.
6. Add 300 $\mu\ell$ of 1.2% fibrin into negative control.
7. Centrifuge at 12,000 rpm for 5 min
8. Transfer supernatant to fresh tube.
9. Measure enzyme activity at 275nm.

► A fibrinolytic unit was defined as the amount of enzyme that gave an increase in absorbance at 275nm equivalent to 1 μg of tyrosine per minute at 30°C.

$$\triangleright \text{Nattokinase activity (unit/100}\mu\ell) = (A_1 - A_0) / 0.0141 \times 1/10 \times D$$

A₀ : Absorbance of negative control at 275nm

A₁ : Absorbance of test sample at 275nm

10 : reaction time

0.0141 : slope from tyrosine standard curve

D : dilution rate of enzyme solution

► Analysis reference ;

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