Vesta

Vesta Pharmaceuticals, Inc

Nutritional Supplements & Nutraceuticals

Nattokinase Analytical Method

Preparation of Test Sample:

1. Dissolve 20 grams of the crude enzyme in 200 ml of 25 mM imidazole- HCL buffer (20 mM $CaCl_2$, pH 7.4).
2. Remove the precipitate by centrifugation at 8600g for 10 min.
3. Fractionate it by adding $(NH_4)_2SO_4$.
4. Dissolve the precipitate got from fractionating in 2 ml of 0.05 M phosphate buffer.
5. Collect the Fractions containing the enzyme activity.

Analytical Test Method:

Add 100? of test sample to 300? of 0.1M Tris-HCl (pH 7.8) containing 10mM $CaCl_2$.

1. Pre-warm the mixture in 30°C water bath for 5 min.
2. Add 300? 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).
3. Incubate the mixture at 30°C for 10 min.
4. To stop the enzyme reaction, add 600? of 0.11M trichloroacetic acid containing 0.22M sodium acetate and 0.33M acetic acid to the mixture.
5. Add 300? of 1.2% fibrin into negative control.
6. Centrifuge at 12,000 rpm for 5 min
7. Transfer supernatant to fresh tube.
8. Measure enzyme activity at 275nm.

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

▷Nattokinase activity (unit/100?) =(A1-A0)/0.0141 ×1/10× D

A_0: Absorbance of negative control at 275nm

A_1: Absorbance of test sample at 275nm

10: reaction time

0.0141: slope from tyrosine standard curve

D: dilution rate of enzyme solution

? **Analysis reference;**
"J. Agric. Food Chem. 2000, 48, 3210-3216 "