ASPARAGINASE FROM *ASPERGILLUS ORYZAE* EXPRESSED IN *A. ORYZAE*

New specifications prepared at the 68th JECFA (2007) and published in FAO JECFA Monographs 4 (2007). An ADI "not specified" was established at the 68th JECFA (2007).

SYNONYMS Asparaginase

SOURCES Asparaginase is produced by submerged fed-batch fermentation of a genetically modified strain of *Aspergillus oryzae* which has a reduced capability for producing secondary metabolites and contains the asparaginase gene derived from *A. oryzae*. The enzyme is isolated from the fermentation broth by filtration to remove the biomass and concentrated by ultrafiltration and/or evaporation. The residual production microorganism is removed from the enzyme concentrate by germ filtration. The final product is formulated using food-grade stabilizing and preserving agents and standardized to the desired activity.

Active principles: Asparaginase

Systematic names and numbers: L-Asparagine amidohydrolase; EC 3.5.1.1; CAS No. 9015-68-3

Reactions catalysed: Hydrolysis of L-asparagine to L-aspartic acid and ammonia.

Secondary enzyme activities: No significant levels of secondary enzyme activities.

DESCRIPTION Light brown liquid

FUNCTIONAL USES Enzyme preparation.
Used in food processing to reduce the formation of acrylamide from asparagine and reducing sugars during baking or frying.

GENERAL SPECIFICATIONS Must conform to the latest edition of the JECFA General Specifications and Considerations for Enzyme Preparations Used in Food Processing.

CHARACTERISTICS

IDENTIFICATION

Asparaginase activity: The sample shows asparaginase activity.
See description under TESTS.

TESTS

Asparaginase activity

Principle

Asparaginase catalyses the conversion of L-asparagine to L-aspartic acid and ammonia. Ammonia is subsequently combined with α-ketoglutarate to form L-glutamic acid. The reaction is catalysed by glutamate dehydrogenase in the presence of NADH, which is oxidized to NAD^+ with the concomitant loss of absorbance measured at 340 nm. The activity of asparaginase is determined by measuring the rate of consumption of NADH under standard conditions (pH=7.00±0.05; 37.0±0.5^0) and is expressed in ASNU units.

One ASNU is defined as the amount of asparaginase that produces 1 micromole ammonia per minute under the conditions of the assay (pH=7.00±0.05; 37.0±0.5^0).

Apparatus

Spectrophotometer (340 nm) with thermostatic control (37.0±0.5^0) and a 1-cm light path.
Water bath with thermostatic control.
Vortex mixer.
Stopwatch

Reagents and solutions

(Note: use deionized water)

Sodium hydroxide 4 M: Weigh 16.0 g NaOH (Merck 106495 or equivalent). Dissolve in water in a 100-ml volumetric flask. Add water to volume and mix until fully dissolved. The solution is stable for 3 months at room temperature.

MOPS buffer, 0.1 M, pH 7, with Triton X-100, 0.1%: Weigh 20.9±0.5 g MOPS (Sigma M-1254 or equivalent) and dissolve in approximately 950 ml of water in a 1000-ml volumetric flask. Adjust the pH to 7.00±0.05 with 4 M NaOH. Add 1.0 ml of 100% Triton X-100 (Sigma T-9284 or equivalent). Add water to volume and mix. The solution must be used on the day of preparation.

L-Asparagine substrate solution: Weigh 0.25±0.02 g of L-asparagine (Sigma A-7094 or equivalent). Transfer to a 25-ml volumetric flask. Add 20 ml of the MOPS buffer with Triton X-100 and stir until dissolved. Add 0.011±0.001 g of NADH (Roche 107 735 or equivalent). Add 0.063±0.005 g of α-ketoglutarate (Sigma K-3752 or equivalent) and at least 2000 units of glutamate dehydrogenase(EC 1.4.1.3) (Fluka 49392 or equivalent). Remove the stirring magnet. Add MOPS buffer with Triton X-100 to volume and mix. The composition of the solution is: L-asparagine, 10 mg/ml; α-ketoglutarate, 2.5 mg/ml; NADH, 0.44 mg/ml; glutamate dehydrogenase, >80 U/ml. The solution is stable for about 2 hours at room temperature.

Control sample solution: Accurately weigh approximately 0.72 g of an

asparaginase preparation with known activity (for example, 1301 ASNU/g; batch 115-11104; expiration date January 2026; available from Novozymes A/S). Transfer to a 100-ml volumetric flask and add the MOPS buffer with Triton X-100 to volume. Mix until fully dissolved.

Blank: MOPS buffer, 0.1 M, pH 7, with Triton X-100, 0.1%.

Test sample solution: Accurately weigh at least 1 g of the asparaginase product into a 50 ml volumetric flask and add the MOPS buffer with Triton X-100 to volume. Repeat dilution if necessary to obtain the activity of approximately 0.4-1.0 ASNU/ml (corresponds to approximately 0.1-0.25 ABS/min).

Procedure

1. Set the temperature of the spectrophotometer and water bath at 37.0 ± 0.5^0.
2. Set the wavelength at 340 nm and use the MOPS buffer with Triton X-100 as a blank.
3. Equilibrate 2.4 ml of the L-asparagine substrate solution in the water bath for 10 min. Add 0.1 ml of the test or control sample solution, vortex briefly, and transfer 1 ml to a 1-cm quartz cuvette.
4. Place the cuvette in the spectrophotometer and immediately read the absorbance. If the absorbance exceeds 2.3, continue the assay. If the absorbance is below 2.3, prepare a new substrate solution. Read the absorbance every 10 sec between 3 and 5 min from the start of the reaction.
5. Plot the absorbance (A) versus time (min) and calculate the slope (ΔA/min).

(Note: Carry out steps 3-5 at least twice for each test or control sample solution. The results should agree within 15%.)

Calculations

Calculate the activity (pASNU/g) of the test or control sample as follows:

$$\text{pASNU/g} = \frac{\Delta\text{A/min} \times \text{Tv} \times \text{Dv} \times \text{F}}{\text{Sv} \times \varepsilon \times \text{d} \times \text{W}}$$

where:

ΔA/min is the absolute value of the decrease of absorbance per min for the test or control sample solution

Tv is the total assay volume (2.5 ml)

Dv is the dissolution volume of the test or control sample (before dilution) (ml)

F is the dilution factor

Sv is the volume of the enzyme solution used in the assay (0.1

ml)

ε is the extinction coefficient of NADH at 340 nm (6.3 ml μmol^{-1} cm^{-1})

d is the light path (1 cm)

W is the weight of the test or control sample (g)

Calculate the ratio (R) of the known-to-calculated activity for the control sample. If R≠1, multiply the activity of the test sample (pASNU/g) by R to obtain the corrected activity of the test sample (ASNU/g):

$$\mathrm{ASNU/g} = \mathrm{R} \times \mathrm{pASNU/g}$$

Notes:
The method is specific for asparaginase activity when up to 6 g/l ammonia is generated in samples with asparaginase activity of 1200 ASNU/g.

Asparaginase activity may vary within ±15% between the replicates of the same sample.