L-Arginine/Urea/Ammonia, UV method

Catalogue number: AK00171, 50 tests of each

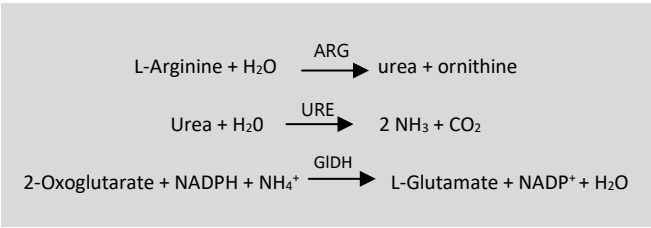
Application

This rapid and simple stereo-specific enzymatic method is used for the simultaneous determination of L-arginine, urea and ammonia (yeast available nitrogen, YAN) in foodstuffs and in the wine industry (grape juice, fermenting must and fermenting wine). This kit is suitable for both manual and micro-volume formats.

Introduction

The determination of YAN (L-arginine, urea and ammonia) is an indicator of the amount of nitrogen available for yeast growth. YAN comprises three components: free ammonium ions, amino nitrogen from free amino acids and from the side chain of L-arginine. As the concentrations of these compounds vary widely, each compound must be determined for the accurate calculation of YAN, which is possible by using this Nzytech's kit. In wine industry, the YAN can be managed by addition of nutrient supplements to grape juice prior to/during fermentation. Nzytech has also analytical kits for the determination of ammonia (AK000091) and both urea and ammonia (AK000101).

Principle



The amount of $NADP^+$ formed through the combined action of arginase (ARG), urease (URE) and glutamate dehydrogenase (GlDH), measured at 340 nm, is stoichiometric with the amount of L-arginine, urea and ammonia in sample volume.

Specificity

The determination is specific for L-arginine, urea and ammonia.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 absorbance units and a sample volume v = 2.00 mL. This corresponds to L-arginine, urea and ammonia concentrations of 0.093, 0.031 and 0.018 mg/l sample solution, respectively, when measured at 340 nm. The detection limit of 0.37, 0.13 and 0.07 mg/l for L-arginine, urea and ammonia, respectively, is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume v = 2.00 mL.

Linearity and precision

Linearity of the determination exists from 1.0 to 35 µg of L-arginine/assay, 0.3 to 14 µg urea/assay and 0.2 to 7 µg ammonia/assay. In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. The relative standard deviation ("coefficient of variation") is approx. 1 to 3%.

Kit composition

Solution 1. TEA buffer (25 mL, 0.5 M, pH 8.0). Store at 2°C to 8 °C.

Tablets 2. 125 tablets of NADPH supplied in a plastic vial containing desiccant. Allow this container to warm to room temperature (in the presence of a desiccant if possible) before opening to remove tablets. Store desiccated at 2°C to 8 °C. (Long-term storage at -30°C to -15 °C)

Add 5 tablets per mL of solution 1 (Solution 1+2) to a test tube and stir intermittently over 2-3 min. Use 0.5 mL of Solution 1+2 per assay, including blank reaction.

Suspension 3. Glutamate dehydrogenase (GlDH) is provided in 2.5 M lithium sulphate (EC 1.4.1.2; 1.1 mL, 915 U/mL). Store at 2°C to 8 °C. Swirl bottle before use.

Suspension 4. Urease is provided in 2.5 M lithium sulphate (EC 3.5.1.5; 1.1 mL, 3000 U/mL). Store at 2°C to 8 °C. Swirl bottle before use.

Suspension 5. L-Arginase is provided in 2.5 M lithium sulphate (EC 3.5.3.1; 1.1 mL, 8300 U/mL). Store at 2°C to 8 °C. Swirl bottle before use.

Solution 6. Ammonia standard solution (5 mL, 0.04 mg/mL) in 0.02 % (w/v) sodium azide. Store at 2°C to 8 °C. This standard should be used when there is doubt about the method accuracy.

Powder 7. L-Arginine standard powder (~2 g). Store desiccated at 2°C to 8 °C. volumetric flask, fill to the mark with distilled water and mix thoroughly. This control solution is stable for ~3 months at -20 °C.

Safety

The reagents used in the simultaneous determination of L-arginine, urea and ammonia are not hazardous materials (see Hazardous Substances Regulations). However, the general safety measures that apply to all chemical substances should be followed.

Procedure (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 2.62 mL (ammonia); 2.64 mL (urea); 2.66 mL (L-arginine)

Sample solution: 0.2 – 7 µg of ammonia, 0.3 – 14 µg of urea and 1 – 35 µg of L-arginine per cuvette (in 0.1 – 2.0 mL sample volume)

Read absorbance against air (without cuvette in the light path), or against water

Pipette into cuvettes (mL)	**Blank**	**Sample**
Distilled water (at ~25 °C)	2.10	2.00
Sample (can vary 0.10-2.00 mL)	-	0.10
Solution 1+2 (NADPH/TEA buffer)	0.50	0.50
Mix, measure the absorbance of the solutions (A1) after ~2 min and start the reaction by addition of		
Suspension 3 (GlDH)	0.02	0.02
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (~4 min)*. Then add		
Suspension 4 (Urease)	0.02	0.02
Mix, measure the absorbance of the solutions (A3) at the end of the reaction (~7 min)*; **		
Suspension 5 (L-Arginase)	0.02	0.02
Mix, measure the absorbance of the solutions (A4) at the end of the reaction (~15 min)*; **		

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

* if necessary, continue to read the absorbance at 5 min intervals until the reactions ends.

** If the final absorbance is below 0.5 AU, please dilute the sample accordingly and repeat the assay.

Calculation

Determine the absorbance difference for both blank and sample (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{Ammonia}$. Determine the absorbance difference for both blank and sample (A2-A3). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining ΔA_{Urea}. Finally, determine the absorbance difference for both blank and sample (A3-A4). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{L.arginine}$.

The concentrations of ammonia (g/L), urea (g/L) and L-arginine, based on the ε of NADH at 340 nm (6300 L×mol-1×cm-1), are calculated as follows:

$$C\ (ammonia) = 0.07082x\ \Delta A_{Ammonia} \quad [g/L]$$

$$C\ (urea) = 0.2517x\ \Delta A_{Urea} \quad [g/L]$$

$$C\ (L\text{-}arginine) = 0.3678x\ \Delta A_{L\text{-}arginine} \quad [g/L]$$

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

The YAN (mg nitrogen/L) can be calculated from ammonia, urea and L-arginine concentrations, using the following equation:

$$YAN = 1000 \times \left[\frac{\text{g ammonia/L} \times 14.01}{17.03} + \frac{\text{g urea/L} \times 28.02}{60.06} + \frac{\text{g L-arginine/L} \times 42.03}{174.21} \right]$$

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the "Alternative Procedures Brochure", available on the NZYTech website.

Interferences

If the conversion of L-arginine, urea and ammonia has been completed within the time specified in the assay, it can be generally concluded that no interference has occurred. However, this can be further checked by adding L-arginine (~10 µg in 0.1 mL) to the cuvette on completion of the reaction. A significant decrease in the absorbance value should be observed. Interfering substances in the sample being analysed can be identified by including an internal standard. Although tannins in fruit juice can lead to the inhibition of some GlDH, the enzyme used in this kit does not have this limitation.

General information on sample preparation

The amount of L-arginine, urea and ammonia present in the cuvette should range between 1 and 35 µg, 0.3 and 14 µg, and 0.2 and 7 µg, respectively. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield L-arginine, urea and ammonia concentrations between 50 and 400 mg/L, 20 and 140 mg/L, and 10 and 80 mg/L, respectively. However, the sample volume can range from 0.10 to 2.00 mL, by replacing water.

To implement this assay use clear, slightly coloured and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (*e.g.* by filtration); adjust acid samples, which are used undiluted for the assay, to approx. pH 8 by adding 2 M NaOH; measure "coloured" samples (if necessary adjusted to approx. pH 8) against a sample blank; treat "strongly coloured" samples that are used undiluted or with a higher sample volume with 0.2 g PVPP/10 mL of sample; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water at 60 °C.

Examples of sample preparation

Determination of L-arginine and ammonia in grape juice

The concentration of L-arginine and ammonia in white and red grape juice can be determined usually without any sample treatment, except filtration and dilution, if necessary. However, no dilution is usually required and a sample volume of 25-100 µL is generally satisfactory.

Determination of L-arginine, urea and ammonia in fermenting must and wine

The concentration of L-arginine, urea and ammonia in white and red fermenting must and wine can be determined usually without any sample treatment, except filtration and dilution, if necessary. However, no dilution is usually required and a sample volume of 25-100 µL is generally satisfactory.

References

Orduna, M. (2001). Quantitative determination of L-arginine by enzymatic end-point analysis. J Agric Food Chem, 49, 549-552.

Recommendations

A method with less interference than chemical methods recommended/approved by the:

- German and Dutch food law (urea and ammonia determinations);

- Recommended by the Mitteleuropäische Brautechnische Analysenkommission (MEBAK) (Central European Commission for Brewing Technology) (urea and ammonia determinations).

V2201

Certificate of Analysis

Test	Criteria	Result
Test Performance	Reaction completed within time stated	Meets specification
	Target value for recommended standard material +/- 10%	Meets specification
Blank reaction absorbance	+/- 10% of the blank value	Meets specification

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

For research use only

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications.

Estrada do Paço do Lumiar, Campus do Lumiar - Edifício E, R/C, 1649-038 Lisboa, Portugal Tel.:+351.213643514 Fax: +351.217151168

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