L-Glutamine/Ammonia, UV method

Catalogue number: AK00111, 50 tests of each

Application

This rapid and simple specific enzymatic method is used for the simultaneous determination of L-glutamine and ammonia (ammonium ions) in cell culture media and foodstuffs such as powdered dietary supplements, bakery products and fruit and vegetable products.

Introduction

L-Glutamine is an essential nutrient of cell culture media. However, the incorporation of this labile amino acid in growth media leads to its spontaneous break down to L-glutamate and free ammonium ions. The released ammonium ions are very toxic to cells. To overcome these issues, either pre-formulated growth media are used strictly within their recommended shelf-lives or L-glutamine is added just before use. In either case, monitoring of L-glutamine and ammonia is frequently performed both prior to, and during culturing. Related analytical kits are also available from Nzytech for ammonia (AK00091) and urea/ammonia (AK00101).

Principle

$$\text{L-Glutamine} + H_2O \xrightarrow{\text{GLT}} \text{L-Glutamate} + NH_4^+$$

$$\text{2-Oxoglutarate} + \text{NADPH} + NH_4^+ \xrightarrow{\text{GlDH}} \text{L-Glutamate} + NADP^+ + H_2O$$

The amount of $NADP^+$ formed through the combined action of glutaminase (GLT) and glutamate dehydrogenase (GlDH), measured at 340 nm, is stoichiometric with the amount of L-glutamine and ammonia in sample volume.

Specificity

The determination is specific for L-glutamine and ammonia.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.005 absorbance units and a sample volume v = 1.00 mL. This corresponds to a L-glutamine concentration of 0.27 mg/L sample solution, when measured at 340 nm. The detection limit of 0.54 mg/L for L-glutamine is derived from the absorbance difference of 0.010 (340 nm) and a maximum sample volume v = 1.00 mL.

Linearity and precision

Linearity of the determination exists from 1 µg to 40 µg L-glutamine/assay. In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur for L-glutamine. The relative standard deviation ("coefficient of variation") is approx. 1 to 3% for L-glutamine.

Kit composition

Solution 1. Sodium acetate buffer (11 mL, 0.1 M, pH 4.9) plus sodium azide (0.02 % w/v) as a preservative. Store at 4 °C.

Solution 2 (2×). TEA buffer (25.5 mL, 0.5 M, pH 8.0) plus imidazole (200 mM), 2-oxoglutarate (40 mM) and sodium azide (0.02 % w/v) as a preservative. Store at 4°C.

Tablets 3. 155 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 -20 °C.

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

Suspension 4. Glutaminase is provided in 2.5 M lithium sulphate (EC 3.2.1.5; 1.1 mL, 2500 U/mL). Store at 4 °C. Swirl bottle before use.

Suspension 5. Glutamate dehydrogenase (GlDH) is provided in 2.5 M lithium sulphate (EC 1.4.1.2; 2.2 mL, 915 U/mL). Store at 4 °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 4 °C.

This standard should be used when there is doubt about the method accuracy.

Powder 7. L-Glutamine control powder (~2 g). Store at 4 °C. This standard should be used when there is doubt about the method accuracy.

Accurately weigh 0.30 g of L-glutamine into a 1 L volumetric flask, fill to the mark with distilled water and mix thoroughly. Stable for ~ 2 months at -20°C.

Safety

The reagents used in the determination of L-glutamine 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 ºC

Final volume: 2.34 mL

Sample solution: 0.2 – 7.0 µg of ammonia per cuvette or 1 – 40 µg of L-glutamine (in 0.1 – 2.0 mL sample volume)

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

	Ammonia		**L-Glutamine**	
Pipette into cuvettes (mL)	Blank	Sample	Blank	Sample
Solution 1	-	-	0.20	0.20
Sample (from 0.10-1.00 mL)	-	0.10	-	0.10
Suspension 4 (Glutaminase)	-	-	0.02	0.02
Mix and incubate for 5 min at room temperature. Then add				
Distilled water (at ~25 °C)	1.82	1.72	1.60	1.50
Solution 2+3 (NADPH/TEA)	0.50	0.50	0.50	0.50
Mix, measure the absorbance (A1) after ~4 min. Then add				
Suspension 5 (GlDH)	0.02	0.02	0.02	0.02
Mix, measure the absorbance of the solutions (A2) at the end of the reaction (approx. 4 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 absorbances at 1 min intervals until the reactions ends.

Calculation

Determine the absorbance difference for both blanks and samples (A1-A2). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining ΔA from the analyte:

$$\Delta A_{Ammonia} = (A1\text{-}A2)_{Ammonia\ sample} - (A1\text{-}A2)_{Ammonia\ blank}$$

$$\Delta A_{L\text{-}glutamine+ammonia} = (A1\text{-}A2)_{L\text{-}glutamine\ sample} - (A1\text{-}A2)_{L\text{-}glutamine\ blank}$$

$$\Delta A_{L\text{-}glutamine} = \Delta A_{L\text{-}glutamine+ammonia} - \Delta A_{Ammonia}$$

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

$$C\ (ammonia) = 0.06325 \times \Delta A_{Ammonia} \quad [g/L]$$

$$C\ (L\text{-}glutamine) = 0.5427 \times \Delta A_{L\text{-}glutamine} \quad [g/L]$$

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.

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-glutamine and ammonia has been completed within the time specified in the assay (approx. 5 min), it can be generally concluded that no interference has occurred. However, this can be further checked by adding L-glutamine (~7 µg in 0.1 mL) or ammonia (~4 µg in 0.1 mL) to the cuvette on completion of the

reaction. A significant decrease in the absorbance should be observed. Interfering substances in the sample being analysed can be identified by including an internal standard.

General information on sample preparation

The amount of ammonia and L-glutamine present in the cuvette should range between 0.2 and 7 µg, and 1 and 40 µg, respectively. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield ammonia and L-glutamine concentrations between 2 and 70 mg/L, and 10 and 400 mg/L, respectively. However, the sample volume can range from 0.10 to 1.00 mL, by replacing water (ammonia and L-glutamine range from 0.20 to 70 mg/L and 1.0 to 400 mg/L, respectively).

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 7.4 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 ammonia and L-glutamine in cell culture media

The concentration of ammonia and L-glutamine in cell culture media or supernatants can be determined without any sample treatment (except clarification by centrifugation / filtering or dilution according to the dilution table, if necessary). Usually, no clarification or dilution is required, and a sample volume of 0.1 mL is satisfactory.

Determination of ammonia and L-glutamine in powdered dietary supplements

The concentration of ammonia and L-glutamine in dietary supplements, such as pharmaceutical grade L-glutamine, can be determined by weighing 5 g of material into a 100 mL volumetric flask. After addition of approx. 60 mL of distilled water, stir the contents until fully dissolved or suspended, and fill up to the mark with distilled water. Mix and, if necessary, filter through Whatman No. 1 filter paper. Use the clear filtrate, with the appropriate dilution, if necessary. In general, for pharmaceutical grade L-glutamine, a further dilution of 1:100 and sample volume of 0.1 mL are satisfactory.

Determination of ammonia and L-glutamine in fruit and vegetable products (*e.g.* potato juice).

Weigh approx. 10 g of material into a 100 mL bottle, add 20 mL of perchloric acid and homogenise for 2 min. Quantitatively transfer 40 mL to a glass beaker and adjust the pH to approx. 8.0 using 2 M KOH. Quantitatively transfer to a 100 mL volumetric flask and adjust to the mark with distilled water. Store on ice for 20 min to allow the precipitatiom of potassium perchlorate and fat separation. Filter, discarding the first 3-5 mL, and use the clear filtrate for the assay. Usually, no further dilution is required and a sample volume of 0.2 mL is satisfactory.

References

Lund, P. (1990). L-Glutamine and L-Glutamate. In Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol. VIII, pp. 357-363,VCH Publishers (UK) Ltd., Cambridge, UK.

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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.

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L-Glutamine and Ammonia, UV method

Alternative Procedures Brochure

Introduction

This kit has been developed to work in cuvettes with a standard pathlength of 1 cm, as described in the respective "Product Brochure". However, it can be adapted for use in 96-well microplates or in auto-analysers (micro-volume formats) with minimal assay optimisation. 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 the standard cuvette pathlength of 1 cm. Thus, to perform the calculation of the amount of analyte in the samples under analysis follow one of the three strategies described in the section below.

Strategies for analyte calculation

Auto-analysers use reaction volumes of approximately 0.315 ml and pathlengths from 4 to 8 mm, which is similar to a standard 96-well microplate in which the same reaction volume would have a pathlength of 6 or 7 mm (similar assay volumes). Therefore, in both formats (96-well microplate and auto-analysers systems), the calculation of the analyte must be done by one of the three possible methods described below:

1. Using the pathlength conversion factor

This is the easiest method to perform the calculation of the analyte. However, it requires a microplate reader with pathlength conversion capacity, i.e., the apparatus can detect the pathlength of each well and convert the individual readings to a 1 cm pathlength (cuvette format). In the case of auto-analysers, the absorbance readings should be directly converted to a 1 cm pathlength. This will allow the calculation of the analyte content as described in the "Product Brochure", provided with the kit and available at the NZYTech website.

2. Using one standard curve

In this method, it is necessary to perform a standard curve of the analyte on each microplate that contains the test samples, or in the auto-analyser, and calculate the result from the standard curve of analyte concentration *vs.* absorbance. The standard curve can be performed by using the control solution provided in the kit.

3. Using two standard curves

The most complicated method is to perform standard curves of the analyte in both the cuvette format (i.e. with a 1 cm of radiation pathlength) and the 96-well microplate or auto-analyser formats, and use these results to obtain a mean conversion factor between the cuvette procedure values and the alternative procedure values. The standard curves can be performed by using the control solution provided in the kit.

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

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