3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Ammonia Assay Kit

Catalog Number **AA0100**
Storage Temperature 2–8 °C
DO NOT FREEZE

TECHNICAL BULLETIN

Product Description

This kit is for the quantitative, enzymatic determination of ammonia in food and biological samples. Ammonia reacts with α-ketoglutaric acid (KGA) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of L-glutamate dehydrogenase (GDH) to form L-glutamate and oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$), as follows:

$$KGA + NH_4^+ + NADPH \xrightarrow{GDH} L\text{-Glutamate} + NADP^+ + H_2O$$

The decrease in absorbance at 340 nm, due to the oxidation of NADPH, is proportional to the ammonia concentration. L-Glutamate dehydrogenase reacts specifically with ammonia. The Ammonia Assay Kit may be used to determine ammonia concentrations in the range of 0.2–15 µg/ml.

Components

Sufficient reagents are provided for 100 assays.

Ammonia Assay Reagent 10 vials
(Catalog Number A0853)
The dry reagent contains α-ketoglutaric acid, NADPH, buffers, stabilizers, and nonreactive fillers. The reagent should be stored at 2–8 °C. It should be discarded if the vials exhibit caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution, or if the reconstituted solution appears turbid or yellow in color.

L-Glutamate Dehydrogenase, 1 vial
from bovine liver
(Catalog Number G2294)
The enzyme is supplied as a solution in 50% glycerol. The enzyme solution should be stored at 2–8 °C. DO NOT FREEZE.
This reagent is stable for at least 1 year at 2–8 °C.
Activity: ≥525 units/ml
Unit Definition: One unit will reduce 1.0 µmole of α-ketoglutaric acid to L-glutamate per minute at pH 7.3 and 25 °C in the presence of ammonium ions.

Ammonia Standard Solution 1 vial
(Catalog Number A0978)
The ammonia concentration of the standard solution is ~10 µg/ml (~588 µM as ammonium sulfate). The solution should be stored at 2–8 °C and is stable for at least 1 year. To avoid evaporation or ammonia uptake, **the Ammonia Standard Solution should be kept capped when not being used**. The Ammonia Standard Solution is used to ensure assay reliability. It is not necessary to run the standard to determine the ammonia concentration of samples.

Equipment Required but Not Provided

- Spectrophotometer suitable for measuring absorbance at 340 nm
- Cuvettes
- Pipettes capable of accurately dispensing 10 µl to 1 ml

Precautions and Disclaimer

The Ammonia Assay Kit is R&D use only, not for *in vitro* diagnostic use, drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Ammonia Assay Reagent - Reconstitute each vial with 10 ml of water. After addition of the water, cap the vial and immediately mix several times by inversion. DO NOT SHAKE. The prepared Ammonia Assay Reagent should be kept capped to avoid possible ammonia uptake. The prepared Ammonia Assay Reagent contains ~3.4 mM α-ketoglutaric acid and ~0.23 mM NADPH. It is stable, in the absence of visible microbial growth, for at least 1 day at 18–26 °C, and for 2 weeks at 2–8 °C. The reconstituted reagent may be aliquoted and stored at –20 °C for 6 months. Avoid freeze/thaw cycles.

The L-Glutamate Dehydrogenase solution and the Ammonia Standard Solution are supplied ready-to-use.

Sample preparation - Samples should be clear and colorless. Adjust the pH to 7–8.

Liquid samples should be diluted with water to an ammonia concentration of 0.20–15 µg/ml (0.1 ml final sample volume). Filter or deproteinize sample solutions, if necessary, to clarify. Sample solutions that are strongly colored and have a low ammonia concentration should be decolorized. Carbonated or fermented products must be degassed.

Solid samples should be weighed to nearest 0.1 mg. Extract the solid sample with water. Dilute the extract with water to an ammonia concentration of 0.20-15 µg/ml (0.1 ml final sample volume). Filter or deproteinize solution, if necessary, to clarify.

Procedure

Any compounds present in the sample that react with $NADP^+$/NADPH under the conditions of this assay will result in inaccurate results for determination of the ammonia concentration. Samples containing protein or protein fragments that liberate ammonia from amino acids will result in falsely high values of ammonia. L-Glutamate dehydrogenase is inhibited by heavy metals such as copper, silver, mercury, iron, and zinc. High concentrations of tannins in the sample will slow the reaction due to inhibition of the L-glutamate dehydrogenase.

Make sure the work area is free from ammonia sources.

1. Pipette the following solutions into appropriately marked cuvettes.

Cuvette	Ammonia Assay Reagent	Ammonia Standard Solution	Sample Volume	Water
Reagent Blank	1.0 ml			100 µl
Test	1.0 ml		100 µl	
Standard	1.0 ml	0.05 ml		

   Sample volume may be varied from 10–200 µl as needed. If the sample volume is changed, make the appropriate adjustments in the calculations.

2. Set the spectrophotometer to 340 nm and the absorbance to zero using water as the reference.
3. Mix the contents in each cuvette and incubate for ~5 minutes at 18–35 °C. Measure the absorbance of each solution at 340 nm.
4. Add 10 µl of L-Glutamate Dehydrogenase solution (Catalog Number G2294) to each cuvette.
5. Mix the contents of each cuvette and incubate for ~5 minutes at 18–35 °C. Then measure the absorbance of each solution at 340 nm.
   Note: The L-glutamate dehydrogenase reaction is typically complete in five minutes. If the absorbance at 340 nm continues to decrease after five minutes, a competitive reaction involving the oxidation of NADPH may be occurring. Continue measuring the absorbance at 1 minute intervals until the rate of decrease is constant for 2 minutes. From this constant rate of decrease, calculate the change in absorbance for 5 minutes due to the competitive reaction [ΔA_{340}(CR)]. When calculating the ammonia concentration, ΔA_{340}(CR) must be subtracted from ΔA_{340}(Test).

Calculation:

Determine ΔA_{340} for the Reagent Blank, Test, and Standard. For each: $\Delta A_{340} = A_{Initial} - A_{Final}$

$\Delta(\Delta A_{340})$Test or Standard

= ΔA_{340}(Test or Standard) – ΔA_{340}(Blank)

mg of NH_3/ml of original sample

$$= \frac{(A)\,(TV)\,(MW\ of\ Ammonia)\,(F)}{(\varepsilon)\,(d)\,(SV)\,(Conversion\ Factor\ for\ \mu g\ to\ mg)}$$

$$= \frac{(A)\,(TV)\,(17)\,(F)}{(6.22)\,(1)\,(SV)\,(1{,}000)}$$

$$= \frac{(A)\,(TV)\,(F)}{(SV)} \times 0.00273$$

A = $\Delta(\Delta A_{340})$Test or Standard
TV = Total Assay Volume in ml
SV = Sample Volume in ml
MW of Ammonia = 17 g/mole or equivalently 17 μg/μmole
F = Dilution Factor from Sample Preparation
ε = Millimolar Extinction Coefficient for NADPH at 340 nm [$mM^{-1}\ cm^{-1}$ or equivalently (ml/μmoles)(1/cm)]
d = Light path (cm) = 1 cm

Notes:
This kit may be used to determine ammonia concentrations as low as 0.2 μg/ml [$\Delta(\Delta A_{340})$Test = 0.007] and as high as 15 μg/ml [$\Delta(\Delta A_{340})$Test = 0.50].

The relative standard deviation is approximately 1–2%. The Ammonia Standard Solution should be included with each set of assays. If the value obtained with the Ammonia Standard Solution is within 5% of the stated concentration, the test performance is acceptable.

References

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5. Van Anken, H.C., and Schiphorst, M.E., A Kinetic Determination of Ammonia in Plasma. *Clin. Chim. Acta*, **56**, 151-157 (1974).
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