Technical Bulletin

Ammonia Assay Kit

Catalogue number MAK538

Product Description

Ammonia (NH_3) or its ion form ammonium (NH_4^+) is an important source of nitrogen for living systems. It is synthesized through amino acid metabolism and is toxic when present at high concentrations. In the liver, ammonia is converted to urea through the urea cycle. Elevated levels of ammonia in the blood (hyperammonemia) have been found in liver dysfunction (cirrhosis), while hypoammonemia has been associated with defects in the urea cycle enzymes (for example ornithine transcarbamylase).

Simple, direct and automation-ready procedures for measuring NH_3 are popular in research and drug discovery. The Ammonia Assay Kit is designed to directly measure NH_3 and NH_4^+. In this assay, NADH is converted to NAD^+ in the presence of NH_3, ketoglutarate and glutamate dehydrogenase. The decrease in optical density at 340 nm or fluorescence intensity at $\lambda_{em}/\lambda_{ex}$ = 450/360 nm is directly proportionate to the NH_3 concentration in the sample.

The linear detection range of the kit is 24 to 1000 µM ammonia. The kit is suitable for ammonia (NH_3) determination in biological samples such as serum, plasma, urine, saliva, and cell culture.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 20 mL
  Catalogue Number MAK538A
- Enzyme 120 µL
  Catalogue Number MAK538B
- Ketoglutarate 120 µL
  Catalogue Number MAK538C
- NADH Reagent 1 vial
  Catalogue Number MAK538D
- Standard (20 mM) 400 µL
  Catalogue Number MAK538E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates or black flat bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- Dounce Tissue Grinder Set (Catalogue Number D9063 or equivalent)
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate all reagents to room temperature prior to assay.

NADH Reagent: Reconstitute the vial with 1000 µL purified water (final conc. 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored at -20 °C.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Serum and plasma Samples can be assayed directly.

Cell culture media should be diluted 5-10-fold in purified water prior to assay.

Solid Samples can be extracted by homogenization in purified water and filtered, centrifuged or, if necessary, deproteinized to remove any undissolved material. Samples should be clear and colorless with pH adjusted to 7 - 8.

Standard Curve Preparation

1. Prepare 300 µL of 1000 µM Standard by mixing 15 µL of the Standard (20 mM) with 285 µL of purified water.
2. Prepare Standards in 1.5 mL centrifuge tubes with purified water according to Table 1.

Table 1.
Preparation of Standards

Well No.	1000 µM Standard	Purified Water	NH_3 (µM)
1	100 µL	0 µL	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	0 µL	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate for the colorimetric assay or a black, flat bottom 96-well plate if performing the fluorometric assay.

Note: The fluorometric assay uses the same Standards set up.

Working Reagent Preparation

For each well, prepare 190 µL of Working Reagent in Table 2.

Table 2.
Preparation of Working and Blank Reagent

Reagent	Working Reagent	Blank Reagent (No Enzyme)
Assay Buffer	180 µL	180 µL
Reconstituted NADH Reagent	8 µL	8 µL
Ketoglutarate	1 µL	1 µL
Enzyme	1 µL	-

Assay Reaction

1. Transfer 20 µL of each Sample into two separate wells. One will serve as a sample blank well (R_{Blank}). The other as the sample well (R_{Sample}).
2. For each Standard and Sample well add 180 µL of the Working Reagent to each well.
3. Add 180 µL of the Blank Reagent to the Sample blank wells.
4. Tap plate to mix.
5. Incubate for 30 minutes at room temperature.

Measurement

Measure the optical density (OD) at 340 nm for colorimetric assay or measure fluorescence at λ_{em} = 450 nm/λ_{ex} = 350-360 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the blank reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against the standard concentrations.
3. Determine the slope and calculate the NH3 concentration of Samples using the below equation:

$$\text{Ammonia } (\mu M) = \left(\frac{R_{Blank}-R_{Sample}}{\text{Slope}(\mu M^{-1})} \times DF\right)$$

Where:

R_{Sample} = Fluorescence intensity (F) or OD reading of Sample

R_{Blank} = Fluorescence intensity (F) or OD reading of Sample Blank

DF = Sample dilution factor (DF=1 for undiluted Samples)

Note: If the calculated NH3 concentration is higher than 1000 µM, dilute sample in purified water and repeat assay. Multiply result by the dilution factor (DF).

Unit Conversions: 1000 µM NH_3 equals 1.7 mg/dL or 17 ppm.

Figure 1.
Typical Colorimetric Standard Curve

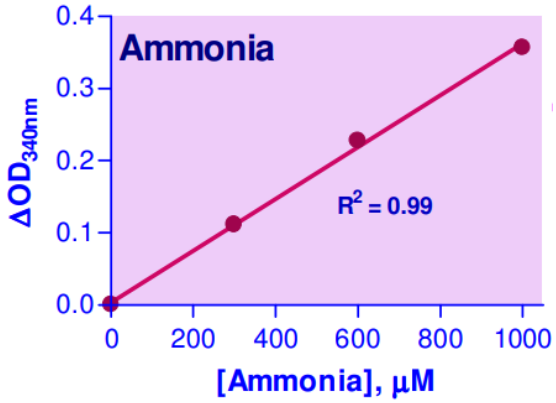
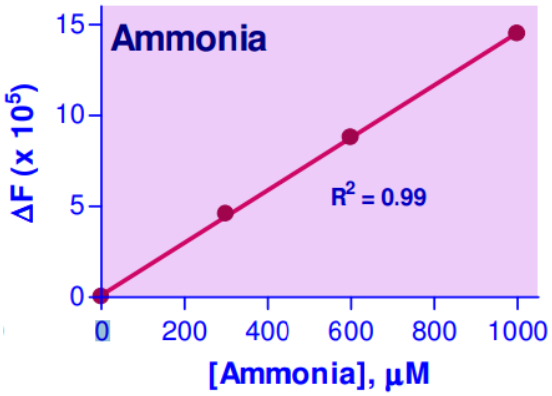


Figure 2:
Typical Fluorometric Standard Curve



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