

EnzyChrom™ Ammonia/Ammonium Assay Kit (ENH3-100)

Quantitative Colorimetric/Fluorimetric Determination of Ammonia

DESCRIPTION

AMMONIA (NH₃) or its ion form ammonium (NH₄⁺) 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 (e.g. ornithine transcarbamylase).

Simple, direct and automation-ready procedures for measuring NH₃ are popular in research and drug discovery. BioAssay Systems' ammonia assay is designed to directly measure NH₃ and NH₄⁺. In this assay, NADH is converted to NAD⁺ in the presence of NH₃, ketoglutarate and glutamate dehydrogenase. The decrease in optical density at 340 nm or fluorescence intensity at λ_{em} = 450/360 nm is directly proportionate to the NH₃ concentration in the sample.

KEY FEATURES

High sensitivity and wide linear range. Use 20 μL sample. Linear detection range 24 to 1000 μM ammonia.

Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of NH₃ within 30 minutes.

APPLICATIONS

Direct Assays: NH₃ in biological samples (e.g. serum, plasma, urine, saliva, cell culture etc).

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 20 mL Enzyme: 120 μL
Ketoglutarate: 120 μL Standard: 400 μL 20 mM NH₄Cl
NADH Reagent: 1 mL

Storage conditions. The kit is shipped chilled. Store all components at -20°C. Shelf life: 3 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample preparation: solid samples can be extracted by homogenization in distilled water (dH₂O) and filtered, centrifuged or, if necessary, deproteinized to remove any undissolved material. Samples should be clear and colorless with pH adjusted to 7 - 8.

Serum and plasma samples can be assayed directly. Cell culture media should be diluted 5-10 fold in dH₂O prior to assay.

Colorimetric Procedure

1. **Standards and Samples.** Equilibrate all components to room temperature. Briefly centrifuge tubes before opening. Prepare a 1000 μM NH₃ Standard Premix by mixing 15 μL of the 20 mM Standard and 285 μL dH₂O. Dilute Standard as follows.

No	Premix + dH ₂ O	Vol (μL)	NH ₃ (μM)
1	100μL + 0μL	100	1000
2	60μL + 40μL	100	600
3	30μL + 70μL	100	300
4	0μL + 100μL	100	0

Transfer 20 μL standards into separate wells of a clear, flat-bottom 96-well plate.

Transfer 20 μL of each sample into two separate wells, one serving as a sample blank well (R_{BLANK}) and one as a sample well (R_{SAMPLE}).

2. **Enzyme Reaction.** For each standard and sample well, prepare Working Reagent by mixing 180 μL Assay Buffer, 1 μL Enzyme, 8 μL NADH Reagent and 1 μL Ketoglutarate. Add 180 μL Working Reagent to the four Standards and the Sample Wells.

Prepare blank control reagent by mixing 180 μL Assay Buffer, 8 μL NADH Reagent and 1 μL Ketoglutarate (No Enzyme). Add 180 μL Blank control reagent only to the Sample Blank Wells.

Tap plate to mix. Incubate 30 min at room temperature.

3. Read OD_{340nm}.

Fluorimetric Procedure

The fluorimetric procedure is the same as for the colorimetric assay, except that a black, flat-bottom 96-well plate is used. After incubation for 30 min at room temperature, read fluorescence intensity at λ_{ex} = 350-360 nm and λ_{em} = 450 nm.

CALCULATION

Subtract the standard values from the blank value (#4) and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the NH₃ concentration of Sample,

$$[\text{Ammonia}] = \frac{R_{\text{BLANK}} - R_{\text{SAMPLE}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

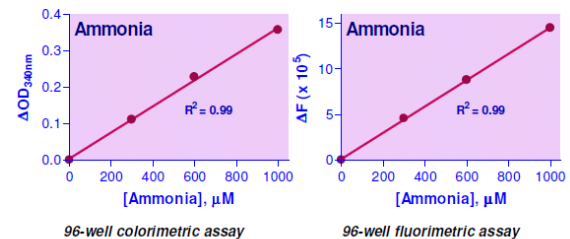
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

Note: if the calculated NH₃ concentration is higher than 1000 μM, dilute sample in dH₂O and repeat assay. Multiply result by the dilution factor *n*.

Conversions: 1000 μM NH₃ equals 1.7 mg/dL or 17 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, and clear flat-bottom 96-well plates and optical density plate reader for colorimetric assays; black flat-bottom 96-well plate and fluorescence intensity plate reader for fluorimetric assays.



LITERATURE

1. Bruce, A.W. et al. (1978). Two-point determination of plasma ammonia with the centrifugal analyzer. Clin Chem. 24:782-787.
2. Mondzac, A. et al. (1965). An enzymatic determination of ammonia in biological fluids. J Lab Clin Med. 66:526-531.
3. Seligson, D. and Hirahara, K. (1957). The measurement of ammonia in whole blood, erythrocytes, and plasma. J Lab Clin Med. 49:962-974.