**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 λₘₚ = 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
- **NADH Reagent**: 400 µL

**Storage conditions.** The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt, 3 weeks after reconstitution.

**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**

1. **Reagent Preparation.** Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening. Reconstitute the NADH Reagent tube with 1000 µL dH₂O (final 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored frozen at -20°C. 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.

2. **Enzyme Reaction.** For each standard and sample well, prepare Working Reagent by mixing 180 µL Assay Buffer, 1 µL Enzyme, 8 µL reconstituted 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 reconstituted 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 OD₃400.** 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 λₘₚ = 350-360 nm and λₑₓ = 450 nm.

**CALCULATION**

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

\[ [\text{Ammonia}] = \frac{R_{\text{Sample}} - R_{\text{Blank}}}{\text{Slope (µM)}} \times n \] (µM)

**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**