

Ammonia Assay Kit

(Catalog #K370-100; 100 assays; Store kit at -20°C)

I. Introduction:

Ammonia is an important source of nitrogen for living systems. Nitrogen is required for the synthesis of amino acids, which are the building blocks of protein. Ammonia is a metabolic product which is created through amino acid deamination. It plays an important role in both normal and abnormal animal physiology like normal animal acid/base balance. BioVision developed a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia. In the assay, Ammonia reacts as substrate with compounds in the presence of enzymes to form a product that reacts with the OxiRed probe to generate color ($\lambda_{\max} = 570 \text{ nm}$) which can be easily quantified by plate reader. The kit can detect 1 nmol (~20 μM) of ammonia, much more sensitive than measuring NADPH based ammonia assay.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Ammonia Assay Buffer	25 ml	WM	K370-100-1
OxiRed Probe	1 vial	Red	K370-100-2
Dimethylsulfoxide (DMSO; Dried)	0.4 ml	Brown	K370-100-3
Enzyme Mix (lyophilized)	1 vial	Green	K370-100-4
Converting Enzyme (Lyophilized)	1 vial	Blue	K370-100-5
NH ₄ Cl Standard (100 mM)	100 μl	Yellow	K370-100-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. **All the solution in this kit should be kept capped when not in use to prevent absorption of ammonia from the air.**

IV. Reagent preparation:

Probe: Dissolve in 220 μl DMSO (provided) before use. Store at -20°C, protect from light and moisture.

Enzyme Mix and Converting Enzyme: Dissolve in 220 μl Assay Buffer separately. Aliquot to prevent multiple freeze/thaw cycle. Store at -20°C. Use within two months.

V. Ammonia Assay Protocol:

1. Standard Curve Preparation:

Dilute the Ammonium Chloride standard solution to 1 mM by adding 10 μl of the 100 mM Ammonium Chloride Standard to 990 μl of dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μl into each well individually. Adjust volume to 50 μl /well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Chloride Standard.

2. Sample Preparations:

Tissues (20 mg) or cells (2×10^6) can be homogenized in 100 μl Assay Buffer, centrifuge at 13,000 $\times g$ for 10 minutes to remove insoluble material. Liquid sample can be tested directly. Add 2-50 μl sample to 96 well plate, bring the volume to 50 μl /well with Assay Buffer. For unknown samples, we suggest testing several different doses of samples to make sure the readings are within the standard curve range.

Note: Pyruvate in samples will interfere with the assay. If significant amount of pyruvate is suspected in your sample, set a pyruvate sample control as in step 3. The pyruvate reading can be subtracted from sample readings.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 μl Reaction Mix.

	Sample	Sample Control
Ammonia Assay Buffer	44 μl	46 μl
OxiRed Probe	2 μl	2 μl
Enzyme Mix	2 μl	2 μl
Converting Enzyme	2 μl	0 μl

Add 50 μl of the **Reaction Mix** to each well containing the NH₄Cl Standard and test samples. Add 50 μl Sample Control Mix to pyruvate sample control. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

4. **Measurement:** Measure O.D. 570 nm in a micro plate reader.

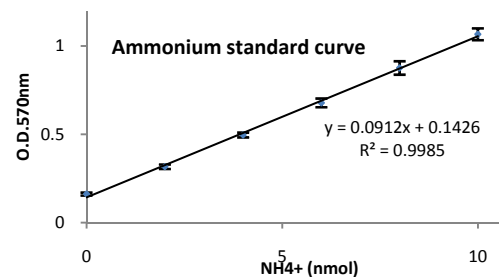
5. **Calculation:** Correct background by subtracting the value derived from the 0 NH₄Cl from all readings (The background reading can be significant and must be subtracted from readings). Subtract the pyruvate Sample Control readings from sample readings. Plot NH₄Cl standard Curve, NH₄Cl concentrations of the samples can then be calculated:

$$C = S_a/S_v \text{ nmol}/\mu\text{l or mM,}$$

where S_a is the sample amount (in nmol) from standard curve.

S_v is the sample volume (μl) added into the wells.

NH₄⁺ Molecular Weight is 18.04 g/mol.



VI. Related Products:

NAD(P)/NAD(P)H Quantification Kit
 Ascorbic Acid Quantification Kit
 Glucose Assay Kit
 Uric Acid Assay Kit
 Pyruvate Assay Kit
 Tryglyceride Assay Kit
 Choline/Acetylcholine Quantification Kit
 Antioxidant Capacity (TAC) Assay Kit
 L-amino Acid Assay Kit
 Ethanol Assay Kit

ADP/ATP Ratio Assay Kit
 Glutathione Detection Kits
 Fatty Acid Assay Kit
 Alanine Assay Kit
 Lactate Assay Kit/ II
 Phosphate Assay Kit
 Hemin Assay Kit
 Glycogen Assay Kit
 Nitric Oxide Assay Kits
 Urea Assay Kit