

Ammonia Colorimetric 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 such as acid/base balance. BioVision provides a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia or Ammonium. In the assay, Ammonia or ammonium is converted to a product that reacts with the OxiRed probe to generate color (OD 570 nm) which can be easily quantified by plate reader. The kit can detect 1 nmol (~20 μM) of ammonia or ammonium, 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 in DMSO	200 μl	Red	K370-100-2A
Enzyme Mix (lyophilized)	1 vial	Green	K370-100-3
Developer	1 vial	Orange	K370-100-4
Converting Enzyme (Lyophilized)	1 vial	Blue	K370-100-5
NH ₄ Cl Standard (10 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:

OxiRed Probe: Warm to room temperature before use. Store at -20°C, protect from light and moisture.

Enzyme Mix, Developer 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 10 mM Ammonium Chloride Standard to 90 μl of ddH₂O, 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 (2x10⁶) can be homogenized in 100 μl Assay Buffer, centrifuge at 13,000 xg 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 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	42 μl	44 μl
OxiRed Probe	2 μl	2 μl
Enzyme Mix	2 μl	2 μl
Developer	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 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 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.

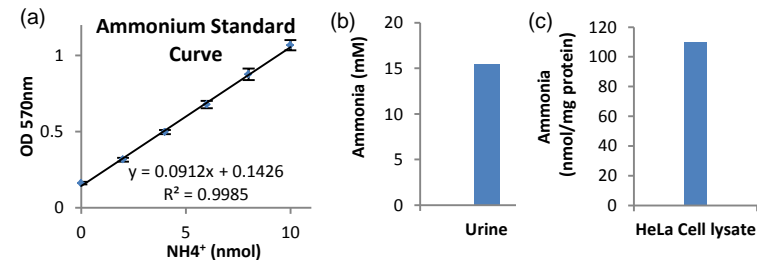


Figure. Ammonia Standard Curve (a). Ammonia concentration in human urine (25 μl, 100 times diluted) (b) and HeLa cell lysate (3 μl, 10.8 mg/ml) (c). Assay was performed according to the kit protocol.

RELATED PRODUCTS:

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|------------------------------------------|----------------------------|
| NAD(P)/NAD(P)H Quantification Kit | ADP/ATP Ratio Assay Kit |
| Ascorbic Acid Quantification Kit | Glutathione Detection Kits |
| Glucose Assay Kit | Fatty Acid Assay Kit |
| Uric Acid Assay Kit | Alanine Assay Kit |
| Pyruvate Assay Kit | Lactate Assay Kit/ II |
| Tryglyceride Assay Kit | Phosphate Assay Kit |
| Choline/Acetylcholine Quantification Kit | Hemin Assay Kit |
| Antioxidant Capacity (TAC) Assay Kit | Glycogen Assay Kit |
| L-amino Acid Assay Kit | Nitric Oxide Assay Kits |
| Ethanol Assay Kit | Urea Assay Kit |

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
<p>Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		