Ammonia Assay Kit

Catalog Number KA0810

100 assays

Version: 03

Intended for research use only

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Table of Contents

Introduction

Background

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. Abnova provide a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia. In the assay, Ammonia is converted to a product that reacts with the OxiRed probe to generate color (O.D. 570 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.

General Information

Materials Supplied

List of component

Component	Amount
Ammonia Assay Buffer	25 mL
OxiRed Probe in DMSO	200 µL
Enzyme Mix (lyophilized)	1 vial
Developer	1 vial
Converting Enzyme (Lyophilized)	1 vial
NH_4Cl Standard (10 mM)	100 µL

Storage Instruction

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.

Assay Protocol

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.

Assay Procedure

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 ddH_2O, 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 ($2x10^6$) 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 NH_4Cl 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.

Data Analysis

Calculation of Results

Correct background by subtracting the value derived from the 0 NH_4Cl from all readings (The background reading can be significant and must be subtracted from readings). Subtract the Sample Control readings from sample readings. Plot NH_4Cl standard Curve, NH_4Cl concentrations of the samples can then be calculated:

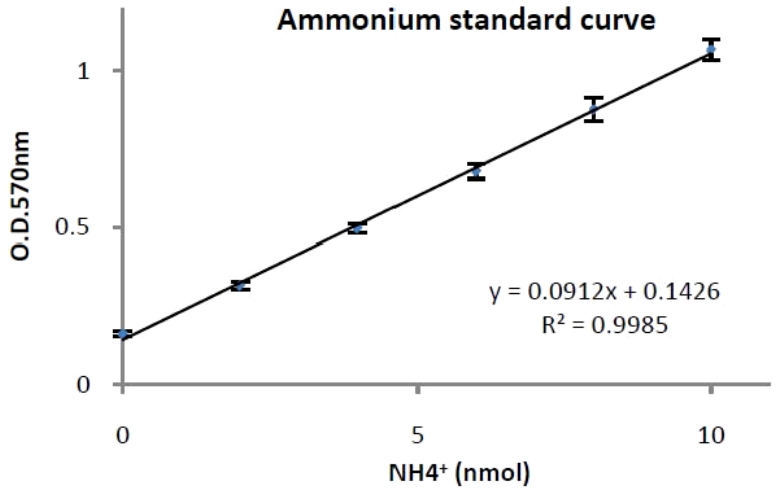
$$C = Sa / Sv \text{ nmol}/\mu\text{L or mM}$$

Where:

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

Sv is the sample volume (µL) added into the wells.

NH_4^+ Molecular Weight is 18.04 g/mol.



Resources

Troubleshooting

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• 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	• 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 (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• 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	• 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 • Use fresh samples or store at correct temperatures until use

Lower/Higher readings in Samples and Standards	• 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	• 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	• 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	• 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	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		