

Ammonia Assay Kit

Catalog Number KA0810

100 assays

Version: 03

Intended for research use only



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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 ₄ Cl 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 μ L of the 10 mM Ammonium Chloride Standard to 90 μ L of ddH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μ L into each well individually. Adjust volume to 50 μ L/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 μ L Assay Buffer, centrifuge at 13,000 xg for 10 minutes to remove insoluble material. Liquid sample can be tested directly. Add 2-50 μ L sample to 96 well plate, bring the volume to 50 μ L/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 μ L of the Reaction Mix to each well containing the NH₄Cl Standard and test samples. Add 50 μ L 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 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:

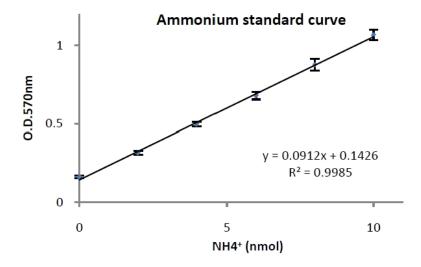
$C = Sa/Sv nmol/\mu L or mM$

Where:

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

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

NH₄⁺ Molecular Weight is 18.04 g/mol.





Resources

Troubleshooting

GENERAL TROUBLESHOOTING GUIDE:

Problems		Cause		Solution
Assay not working	•	Use of ice-cold assay buffer	•	Assay buffer must be at room
	•	Omission of a step in the protocol		temperature
	•	Plate read at incorrect wavelength	•	Refer and follow the data sheet
	•	Use of a different 96-well plate		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	•	Use of an incompatible sample type	•	Refer data sheet for details about
readings	•	Samples prepared in a different		incompatible samples
		buffer	•	Use the assay buffer provided in the kit
	•	Samples were not deproteinized (if		or refer data sheet for instructions
		indicated in datasheet)	•	Use the 10 kDa spin cut-off filter or
	•	Cell/ tissue samples were not		PCA precipitation as indicated
		completely homogenized	•	Use Dounce homogenizer (increase
	•	Samples used after multiple		the number of strokes); observe for
		free-thaw cycles		lysis under microscope
	•	Presence of interfering substance in	•	Aliquot and freeze samples if needed
		the sample		to use multiple times
	•	Use of old or inappropriately stored	•	Troubleshoot if needed
		samples	•	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 • Incorrect volumes used • Use of partially thawed components • Pipetting errors in the standard or incubation fines or pattern for Standard curve • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots • Measured at incorrect wavelength or Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range • 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 • 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 • Use fresh components from the same kit • Recheck calculations after referring the data sheet • Use fresh components from the same kit • 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			
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