

MA-0125

Ammonia Assay

(100 wells, Colorimetric, OD 570 nm, Store at -20°C)

Introduction:

Ammonia is the primary central source of nitrogen for living systems. Ammonia is both a metabolic product and a necessary enzyme substrate for a wide range of reactions. It is created through nitrogen fixation or amino acid deamidation. It's one of the primary contributors to acid/base balance. AkrivisBio's Ammonia Assay provides a simple, sensitive procedure for the accurate determination of ammonia below 1 nmol per well. In the assay, Glutamate dehydrogenase converts NADH in the presence of ammonia plus α -ketoglutarate to NAD which is used to convert lactate to pyruvate. The pyruvate is oxidized forming hydrogen peroxide which is used by peroxidase to convert ADHP to resorufin with intense color (OD 570 nm).

Assay Principle:

- 1 - NADH is converted to NAD by glutamate dehydrogenase in the presence of ketoglutarate and ammonia
- 2 - Lactate dehydrogenase converts lactate to pyruvate in the presence of NAD
- 3 - Pyruvate is oxidized back to lactate with the formation of hydrogen peroxide
- 4 - Peroxidase utilizes the hydrogen peroxide to convert ADHP to resorufin

Assay Components:

Assay Buffer	25 ml	WM	MA-1025-A
ADHP Solution	200 μ l	Red	MA-0125-B
Pyruvate Oxidase/Peroxidase	(lyophilized)	Green	MA-0125-C
Lactate DH/ NADH	(lyophilized)	Orange	MA-0125-D
Glutamate dehydrogenase	(lyophilized)	Blue	MA-1025-E
NH ₄ Cl Standard	100 μ l	Yellow	MA-0125-F

Storage and Handling:

Store all components at -20°C before use. Centrifuge all small vials for a few seconds before opening.

Assay buffer: Warm to room temp before using. Store at +4°C

ADHP Solution: Warm to room temperature before using. Store at -20°C

Pyruvate Oxidase/Peroxidase, Lactate DH, Glutamate DH: Add 220 μ l of DI water to each and dissolve. Once reconstituted, keep on ice while using, store at -20°C. If you plan to use the Assay several times over a period of time, aliquot the enzyme solutions into convenient portions and freeze to prevent multiple freeze/thaw cycles which accelerates enzyme denaturation.

Note: All kit components should be kept capped when not in use to prevent absorption of ammonia from the air. Be particularly cautious in an environment where ammonia is likely to be in the air at measurable concentrations (fermentation labs, HPLC labs)

Assay Protocol:

1. **Standard Curve:** Dilute the Ammonium Chloride standard solution to 0.4 mM by adding 10 μ l of the Ammonium Chloride Standard to 90 μ l of DI H₂O, mix well. Add 0 – 5 – 10 – 15 – 20 – 25 μ l of standard to a series of wells in a 96 well plate. Adjust all well volumes to 50 μ l with Assay Buffer to giving 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Chloride Standard.

2. **Sample Preparation:** Homogenize tissue (10 mg) or cells (10⁶) in 100 μ l Assay Buffer, centrifuge at 16,000 X g (max speed for tabletop centrifuges) for 5 minutes to remove particulates. Add 2-50 μ l of each sample to test wells of a 96 well plate. Adjust all well volumes to 50 μ l with Assay Buffer.

Notes: Sample values must fall into the range of the standard curve. If a sample falls outside, dilute and rerun. Some samples exhibit a significant background. Run samples in pairs with one of the pair used as a background control, omitting glutamate dehydrogenase in the background control. In rare cases, samples exhibit a matrix effect where endogenous compounds interfere, giving a lower OD or RFU/ μ g than the standards. To correct for this, run each sample as a triad: One as a background control (- GDH) One with added known amount of ammonia standard (2 μ g). The third is the original test sample. The difference between the test samples with and without the internal calibrant allows for accurate determination of ammonia in the unspiked sample.

3. **Initiate Reaction:** Prepare sufficient Reaction Mix for the total number of wells to be run. Each well requires 50 μ l of Reaction Mix containing:

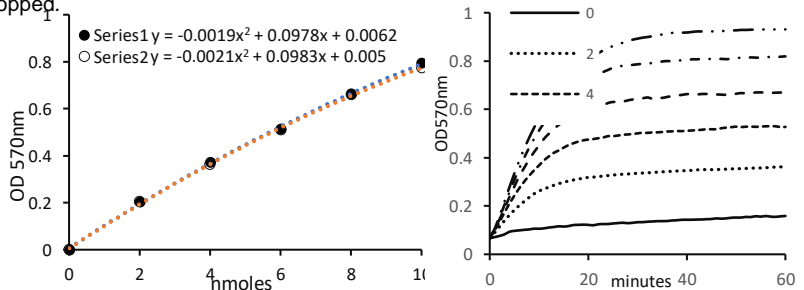
	Sample	Background Control
Assay Buffer	42 μ l	44 μ l
ADHP Solution	2 μ l	2 μ l
Pyruvate Oxidase/Peroxidase	2 μ l	2 μ l
Lactate Dehydrogenase	2 μ l	2 μ l
Glutamate Dehydrogenase	2 μ l	-----

Add 50 μ l of **Reaction Mix** to each well containing Standard or test sample. Add 50 μ l Background Control Mix to paired background controls. Mix well.

4. **Measurement:** Monitor the reaction for 60 min at 37°C using absorbance at 570 nm in a plate reader. When the signal from the standards levels off at a constant value, data acquisition can be stopped.

5. Typical Results:

nmol Standard	Standard Raw Values		Background Corrected Values	
	OD	OD	OD	OD
0	0.1601	0.1587	0	0
2	0.3632	0.3624	0.2031	0.2037
4	0.5321	0.5202	0.3720	0.3615
6	0.6729	0.6687	0.5128	0.5100
8	0.8224	0.8167	0.6623	0.6580
10	0.9543	0.9322	0.7942	0.7735



6. **Calculation:** Subtract the 0 NH₄Cl reading from all standard readings. Plot the corrected absorbance standard curve. Determine the slope of the standard curve (use a linear or polynomial (2^o) fit, whichever fits the data best. The slope will be used for quantifying all unknown samples. Subtract the Background Control readings from the paired unknown readings. Apply the slope of the standard curve to the corrected absorbance values for the unknowns. Correct for sample dilution due to sample processing:

A. nmoles ammonia in well / μ l sample added to well = nmoles ammonia per μ l sample

B. nmoles ammonia per μ l samples X total volume of sample (100 μ l + tissue/cell pellet volume) = total nmoles ammonia in sample

C. total nmoles ammonia in sample/ mg tissue or # of cells/cell mass = nmol ammonia / mg (or /cell # or /cell mass)