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-1025A
ADHP Solution	200 µl	Red	MA-0125B
Pyruvate Oxidase/Peroxidase	lyoph	Green	MA-0125C
Lactate DH/ NADH	lyoph	Orange	MA-0125D
Glutamate dehydrogenase	lyoph	Blue	MA-1025E
NH_4Cl Standard	100 µl	Yellow	MA-0125F

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 µl 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 µl of the Ammonium Chloride Standard to 90 µl of DI H_2O, mix well. Add 0 – 5 – 10 – 15 – 20 – 25 µl of standard to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl 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 (10^6) in 100 µl Assay Buffer, centrifuge at 16,000 X g for 5 minutes to remove particulates. Transfer clear supernatant to a fresh tube. Add 2-50 µl of each sample to test wells of a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer.
Notes: Sample values must be within 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/µg 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 µg). 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 µl of Reaction Mix containing:

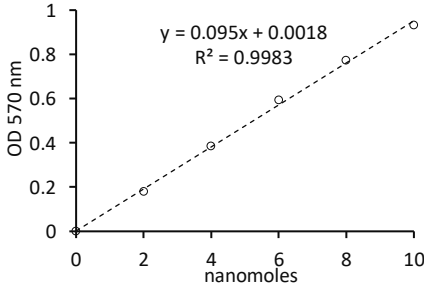
	Reaction Mix	Background Control Mix
Assay Buffer	42 µl	44 µl
ADHP Solution	2 µl	2 µl
Pyruvate Oxidase/Peroxidase	2 µl	2 µl
Lactate Dehydrogenase	2 µl	2 µl
Glutamate Dehydrogenase	2 µl	-------

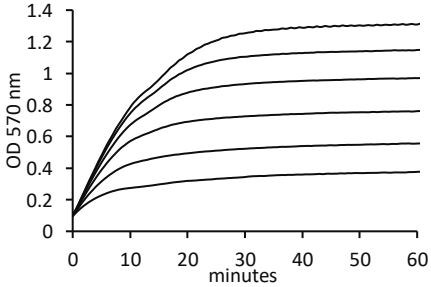
Add 50 µl of **Reaction Mix** to each well containing Standard or test sample. Add 50 µl Background Control Mix to paired background controls. Mix well.

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 OD		Background Corrected Values 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 NH_4Cl reading from all standard readings. Plot the corrected absorbance standard curve. Determine the slope of the standard curve. The slope will be used for quantifying all unknown samples. Subtract the Background Control readings from the paired unknown readings. Divide the background corrected sample absorbance values by the slope of the Standard Curve to get nmoles of ammonia in the wells. To convert back to nanomoles of ammonia in the original samples:
A. Divide the nmoles ammonia in well by the total sample volume added to the well = nmole ammonia per µl sample
B. Multiply nmoles ammonia per µl sample X total volume of supernatant recovered in step 2 above = total nmole ammonia in sample
C. total nmole ammonia in sample/ mg tissue or # of cells/cell mass = nmol ammonia / mg (or /cell # or /cell mass)

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