MA-0118

Urea Assay

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

Background Information:

When urea was first synthesized from inorganic chemicals it invalidated the concept of vitalism wherein it was believed that only living organisms could produce the chemicals from which they are made. Urea is the primary means of nitrogen excretion in mammals. The flow of the -NH_3 group through a myriad of pathways terminates in the formation of urea. Being quite toxic, ammonia is either excreted rapidly, as by fish, converted to uric acid (birds) or urea by mammals and amphibians. Formed in the liver by adding two ammonia molecules to CO_2, urea is virtually non-toxic even at high concentrations, much less so than ammonia from which it is derived. Blood urea nitrogen, a measure of nitrogen in the blood from urea is a useful measure of kidney function. The urea pathway has been found in invertebrates, insects, plants and other organisms. The complex physiology of the mammalian kidney utilizes the high solubility of urea to manipulate osmotic pressure for the reabsorption of water and useful ions in the nephron. AkrivisBio's Urea Assay is a simple, sensitive way to determine urea from a variety of biological samples with a sensitivity less than 0.2 nmol of urea per well.

Assay Principle:

- Urea is hydrolyzed by urease to 2 molecules of ammonia.
- Glutamate dehydrogenase converts the ammonia along with α-ketoglutarate and NADH to glutamate and NAD.
- Lactate dehydrogenase utilizes NAD to convert lactate to pyruvate and NADH (which is fed back into the GDH reaction).
- Pyruvate is oxidized, forming hydrogen peroxide.
- Hydrogen peroxide is utilized by peroxidase to oxidize ADHP to resorufin with strong absorbance at 570 nm.

Assay Components:

Assay Buffer	25 ml	WM	MA-0118A
ADHP Solution	200 µl	Red	MA-0118B
Pyruvate Oxidase/HRP	lyoph	Green	MA-0118C
LDH/GDH	lyoph	Orange	MA-0118D
Urease	lyoph	Blue	MA-0118E
Urea Standard (20 mM)	100 µl	Yellow	MA-0118F

Storage and Handling:

Store all components at -20°C when not in use. Bring assay components to room temperature before use. Briefly centrifuge vials before opening. Keep all solutions capped to prevent absorption of NH_3 from the air.

ADHP Solution: Warm to room temperature before use.

Pyruvate Oxidase/HRP, LDH/GDH, Urease: Add 220 µl Assay Buffer to each and dissolve. Once the enzymes have been reconstituted, 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.

Assay Protocol:

1. **Standard Curve:**

Dilute the Urea Standard to 0.2 mM by adding 10 µl to 990 µl DI H_2O. Add 0 – 5 – 10 – 15 – 20 - 25 µl into a series of wells in a 96 well plate in duplicate. Adjust all well volumes to 50 µl with Assay Buffer to give 0 – 1 – 2 – 3 – 4 – 5 nmol per well of the Urea Standard.

2. **Sample Preparation:**

Homogenize tissue (10 mg) or cells (10^6) in 100 µl Assay Buffer. Centrifuge at 16,000 X g for 10 min to pellet cell debris. Transfer the clear supernatant to a fresh tube. Transfer 1-25 µl of each sample to wells of a 96 well plate. Bring all well volumes to 50 µl per well with Assay Buffer. Sample readings should be within the absorbance range of the standard curve. If any are outside the range, they should be diluted and rerun.

Note: Ammonium ions, NAD^+, $NADP^+$, and pyruvate in the sample can contribute to a background to the assay. A urease negative control will allow for correction for such background.

3. **Initiate Reaction:** Prepare sufficient Reaction Mix for the number of samples and standards to be run. Each well requires 50 µl of Reaction Mix containing:

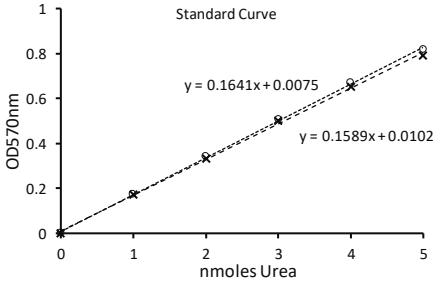
	Reaction Mix	Background Control Mix
Assay Buffer	42 µl	44 µl
ADHP Solution	2 µl	2 µl
Pyruvate Oxidase/HRP	2 µl	2 µl
LDH/GDH	2 µl	2 µl
Urease	2 µl	----

Add 50 µl to each well containing urea standards and test samples. Add 50 µl of the Background Control Mix to all background control wells.

4. **Measurement:** Monitor the reaction in kinetic mode at 37°C for 30 min - 1 hour. When the signal from the standards levels off at a constant value, data acquisition can be stopped. There is a small linear drift which is apparent when the data is viewed in kinetic mode. See section 7 below.

5. **Typical Results:**

nmole	Standard Raw Values		Background Corrected Values	
Standard	OD			
0	0.2534	0.2323	0	0
1	0.4260	0.4046	0.1726	0.1723
2	0.5842	0.5746	0.3308	0.3423
3	0.7512	0.7369	0.4978	0.5046
4	0.8703	0.9143	0.6169	0.6820
5	1.0445	1.0502	0.7911	0.8179



6. **Calculation:** Subtract 0 nmol standard from all other standards. Plot the Standard curve. Determine the slope of the standard curve. The slope of the standard curve defines the OD/nmol obtained from the assay. Correct the raw values for the test samples by subtracting the values for the paired background controls. Divide the background corrected test values by the slope of the standard curve to derive nmoles of urea in the test samples. Correct test samples for amount and dilution to determine the urea content of the original samples:
Total urea of sample = Total volume of test sample / volume of test sample applied to well
Urea Content of Original Sample = Total urea of sample / mass of tissue or # of cells

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