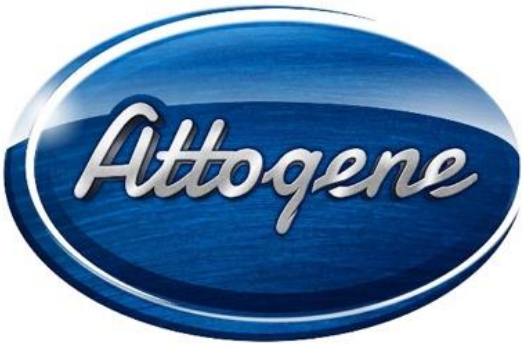

Blood Urea Nitrogen Enzymatic Kit

Catalog Number: EZ2022

For Research Use Only. Not for use in Diagnostic Procedures.

I. Introduction

The Blood Urea Nitrogen Enzymatic Kit is a microplate-based colorimetric assay for the determination of urea in serum samples produced from blood. Blood urea nitrogen (BUN) is an important marker for normal kidney and liver function. Elevation of BUN levels are often an indication of intestinal and kidney obstruction and cardiac failure. Decreased BUN levels are often associated with kidney and liver damage. BUN is also a very useful tool for preclinical investigation of experimental drug formulations and BUN levels are commonly used to monitor and attenuate the toxic effects of experimental drug formulations in rodents.

The Blood Urea Nitrogen Enzymatic Kit uses an enzyme-based assay to determine urea in liquid samples such as serum. The test is based on a highly proven method for urea determination. The Blood Urea Nitrogen Enzymatic Kit contains sufficient materials to test 42 samples in duplicate.

The assay utilizes urease, a metabolic enzyme, to specifically detect urea in serum. The Blood Urea Nitrogen Enzymatic Kit provides rapid, accurate, proven results even in complex liquid mixtures. The limit of detection for the test is 8 ppm urea for serum. The linear range of the assay is 8 – 200 ppm analyte.

The unique features of the kit are:

- Rapid and simple method
- Minimal sample prep
- Highly accurate and reproducible

The kit is designed to be used with a microplate reader. The kit contains urea standards to construct a linear calibration curve and verify assay performance.

2. Procedure Overview

The Blood Urea Nitrogen Enzymatic Kit measures the concentration of urea using the urease enzyme, which converts urea to ammonia.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2\ NH_3$$

The ammonia produced from the urea is then directly detected by a colorimetric chemical reaction.

3. Contents (96 determinations)

Component Name	Volumes	Storage
96 well plate	1 each	RT
Urea Standards: 0ppm, 8ppm, 25ppm, 50ppm, 100ppm, 200ppm	0.4 mL x 6	2 - 8°C
Urease Mix	0.4 g (powder)	2 - 8°C
Alkaline Hypochlorite Solution	20 mL	2 - 8°C

Required materials not included in kit:

- Microplate reader (620nm absorbance filter)
- Deionized water
- Normal saline or PBS (Phosphate-buffered saline, pH 7.4)
- Microcentrifuge
- Microcentrifuge tubes
- Micropipettes: 20µL, 200µL, 1000µL pipettes
- Multichannel pipet (optional)

4. Warnings and Precautions

Attogene strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit.

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

5. Sample Preparation

Serum

- Allow 0.2 – 1 mL blood sample to coagulate in a microcentrifuge tube for 20 minutes at 37°C and then centrifuge for 5 minutes at 9,000 x g.

- Transfer the supernatant (serum) to a clean tube. It is best to test the serum immediately; however, if the samples cannot be tested within 6 hours of collection, store them at 4°C and test no later than 3 days after collection.

- Dilute and mix the serum 1:4 (% v/v) with PBS or normal saline IMMEDIATELY before adding the sample to the well.

6. Urea Determination Test Protocol

Set-up:

Warm up kit reagents to room temperature. Turn on plate reader, allow the light source to warm up, and set absorbance wavelength to 620nm.

Reagent Preparation:

To reconstitute the Urease Mix:

- Add exactly 20 mL of deionized or distilled water to the Urease Mix powder
- Mix by swirling or inverting the bottle 10 times
- Allow contents to dissolve for 10 minutes at room temperature
- Swirl or invert the bottle 10 additional times before use

The Urease Mix is stable for 4 months after reconstitution with water.

IMPORTANT: The reconstituted Urease Mix can be left at room temperature for short periods (30 – 60 minutes) prior to use. Between uses, the reconstituted Urease Mix should be stored at 4°C for up to 4 months.

Discard the Urease Mix 4 months after reconstitution.

Test Procedure:

- Add 5 µL of each standard in duplicate to different wells in the microtiter plate
- Add 5 µL of each diluted serum sample in duplicate to different wells in the microplate plate
- Add 150 µL of Urease Mix solution to each well containing standards or samples
- Mix the solution in the wells for 1 minute by tapping the plate gently or by using a plate shaker, as to not allow any liquid to spill over
- Cover the plate and incubate 15 minutes at room temperature
- Add 150 µL of Alkaline Hypochlorite Solution to each well
- Mix the solution in the wells for 1 minute by tapping the plate gently or by using a plate shaker, as to not allow any liquid to spill over
- Cover the plate and incubate for 10 minutes at room temperature
- Measure the absorbance of each sample in duplicate at 620nm

Note: Dilution factor = 5

Standard Curve Construction:

A calibration curve constructed using the urea standards supplied with the kit is required to determine the urea concentration in the samples.

- Add 5 µL of each standard in duplicate into 150 µL Urease Mix solution in microplate wells. Tap plate gently 3-4 times to mix sample and enzyme. Incubate 15 minutes at room temperature
- Add 150 µL Alkaline Hypochlorite Solution to each well. Incubate for 10 minutes at room temperature
- Measure the absorbance of each sample in duplicate at 620 nm

A plot of average absorbance at 620 nm as a function of urea concentration should provide a tight linear curve. Each of the standard points should be resolved from the other neighboring points.

7. Data Analysis

Calculation of Blood Urea Nitrogen Concentration:

A standard curve can be constructed by plotting the average corrected absorbance obtained from each reference standard against its concentration in ppm.

Calculate the slope and the y-intercept for the line which fits the standard curve data.

The urea concentration (dilution factor = 5) in the well can be determined using the equation:

$$\text{Blood Urea Nitrogen concentration} = \frac{\text{Dilution factor x (Average absorbance} - \text{y-intercept)}}{\text{slope}}$$

About Us:

Attogene is a biotechnology company located in Austin, Texas. Our focus is to enhance health and wellness by offering and developing customer focused Life Science Products domestically and internationally.

Our mission is to:

- Enhance detection technologies
- Enable rapid responses
- Enable impactful research discoveries

Contact Us
3913 Todd Lane, Suite 310
Phone: 512- 333-1330
Email: sales@attogene.com
Web: www.attogene.com
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