MANUAL

Urea Nitrogen (BUN) Assay Kit (Colorimetric)

Catalog #: MA-BUN

ISO 13485:2016

Introduction

Urea is the end product of protein nitrogen metabolism and is the primary vehicle for removing toxic ammonia from the body. It is synthesized in the liver from the ammonia produced from the catabolism of amino acids via the hepatic urea cycle. Urea is transported in the bloodstream to the kidneys where it is excreted in the urine. The rate of urea clearance and elimination through the kidneys correlates with the flow rate; higher flow rates result in greater urea nitrogen clearance. Consequently, the level of circulating urea nitrogen, along with serum creatinine, serves as a primary measure of kidney function. Normal adult Blood Urea Nitrogen (BUN) levels typically range between 7 and 21 mg urea nitrogen per 100 mL blood (mg/dL). Elevated BUN levels (≥ 50 mg/dL), known as azotemia, are associated with acute kidney failure or injury, severe acute pancreatitis, congestive heart failure or gastrointestinal bleeding. Azotemia also can occur due to dehydration, alcohol abuse, or high protein diets. Lower than expected BUN levels are usually less clinically significant but may indicate liver disease or malnutrition, including malabsorption and low protein diets.

Urea Nitrogen (BUN) Detection Assay Kit provides a simple, reproducible, and sensitive tool for measuring urea nitrogen in plasma, serum, cell lysates, urine and other biological liquid samples. This assay utilizes a coupled enzymatic reaction system. First, urea is hydrolyzed by urease to produce ammonia and carbon dioxide. The liberated ammonia reacts with α-ketoglutarate in the presence of NADH to yield glutamate. During this reaction, an equimolar quantity of NADH undergoes oxidation resulting in a decrease in absorbance at 340 nm directly proportional to the urea nitrogen concentration in the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C
Urea Nitrogen Standard	1 vial (210 µl of 20 mg/dL)	2–8 °C
Enzyme Mix Solution	14 ml	2–8 °C
Coenzyme Solution	3 ml	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 37ºC
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Incubator at 37ºC

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, urea levels in human plasma are in the range of 2- 7 mM. Plasma samples can be diluted 1:3 – 1:10 with Sample Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, urea levels in human serum are in the range of 2- 7 mM. Serum samples can be diluted 1:3 – 1:10 with Sample Buffer.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Typically, urea levels in human urine are in the range of 50- 300 mM. Urine samples can be diluted 1:100 – 1:400 with Sample Buffer.

NOTE:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

Reagent Preparation

A. Working Solution

Mix Enzyme Mix Solution and Coenzyme Solution at a volumetric ratio of 5:1 to make the Working Solution. For example, mix 13.5ml of Enzyme Mix Solution and 2.7ml of Coenzyme Solution to prepare sufficient Working Solution for one 96-well plate. Mix well. Protect from light. The Working Solution is stable for 14 days at 4 ^{o}C.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 mg/dL – 5 mg/dL (see Table below),

1. Label 8 microtubes #1 through 8 with the following concentrations: 5, 4, 3, 2, 1, 0.5, 0.25, 0 mg/dL.
2. Pipette the amount of Sample Buffer as described in Table 1 below into labeled tube #1 through #8.
3. Pipette 100 µL 20 mg/dL Urea Nitrogen Standard (provided) into tube #1, Mix thoroughly to make 5 mg/dL Urea Nitrogen Standard.
4. To make the 4 mg/dL standard, pipette 300µL tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly, and used immediately. The diluted standards are stable for eight hours at 4^{o}C.

Labeled Tubes	Urea Nitrogen Standard (µL)	Sample Buffer (µL)	Standard Conc. (mg/dL)	Standard Conc. (µM)
1	100µL	300µL	5	835
2	300µL of Tube #1	75µL	4	668
3	225µL of Tube #2	75µL	3	501
4	150µL of Tube #3	75µL	2	334
5	100µL of Tube #4	100µL	1	167
6	100µL of Tube #5	100µL	0.5	83.5
7	100µL of Tube #6	100µL	0.25	41.75
8	0µL	100µL	0	0

Table 1. Preparation of Urea Nitrogen standards.

Assay Procedure

Each Urea Nitrogen Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Set up a microplate reader or a microplate incubator at 37°C.
2. Prepare Working Solution (See Reagent Preparation, section A), and incubate it at 37°C for at least five minutes.
3. Add 30 µL of the diluted Urea Nitrogen Standards or samples to the 96-well microtiter plate.
4. Initiate the reaction by adding 150 µL of Working Solution to each well (pre-heat reagent at 37°C).
5. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
6. After thirty seconds read and record the absorbance (A1) at 340nm using a plate reader.
7. One minute after the first reading take another reading (A2) at 340nm.
8. Determine the absorbance change between the two readings (A1-A2).

Calculation of Results

Subtract the blanks

Calculate the mean absorbance for each set of duplicate samples, and Sample Buffer blank (Standard 0 mg/dL). Determine the absorbance change ΔA between the two readings (A1, A2). Subtract the absorbance change of Sample Buffer blank for each sample, this is the corrected absorbance change.

$\Delta A_{Sample} = A1_{Sample} - A2_{Sample}$ $\Delta A_{Blank} = A1_{Blank} - A2_{Blank}$

$\Delta A' = \Delta A_{Sample} - \Delta A_{Blank}$

Plotting the standard curves

Make a plot of corrected absorbance change at 340nm as a function of Urea Nitrogen concentration.

Determination of sample Urea Nitrogen concentration

Urea Nitrogen (mg/dL) = $\frac{\Delta A_{Sample} - \Delta A_{Blank}}{Slope} \times DF$

ΔA_{Sample} = absorbance change (ΔA) of the Sample

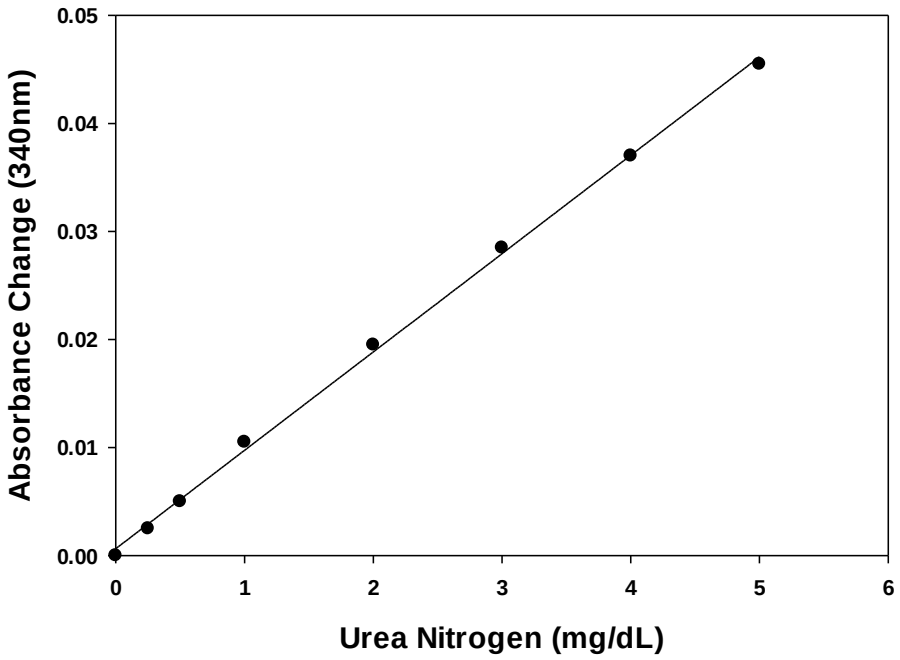
ΔA_{Blank} = absorbance change (ΔA) of the Blank (Standard 0 mg/dL)

Slope is from the plot of Urea Nitrogen concentration vs. Absorbance change, as shown in Typical data below.

DF = Sample Dilution factor (DF = 1 for undiluted samples)

Note: If the calculated Urea Nitrogen concentration of the sample is higher than 5 mg/dL, dilute the sample in Sample Buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of Urea Nitrogen is about 0.138 mg/dL.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 6.7%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 9.9%.

This product is for research use only.