StressMarq Biosciences INC.

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StressXpress®

Blood Urea Nitrogen Detection Kit

Catalog# SKT-213 (2 Plate kit)

Quantitative colorimetric measurement of urea nitrogen

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-213A	Clear 96 well Plates	2 plates	-
SKC-213B	Blood Urea Nitrogen Standard (Urea)	250 µL	Urea Nitrogen at 214mg/dL (100 mg/dL BUN)
SKC-213C	Color Reagent A	15 mL	An acidic solution of Color Reagent A. CAUTION: CAUSTIC
SKC-213D	Color Reagent B	15 mL	An acidic solution of Color Reagent B. CAUTION: CAUSTIC
SKC-213E	Urease Enzyme	2 vials	Lyophilized Urease

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Color Reagents A and B are both strong acid solutions and should be handled like any laboratory acid.

Storage

All reagents, with the exception of the Blood Urea Nitrogen Standard and the Urease Enzyme, are stable as supplied at 4°C. The standard and urease enzyme should be stored at -20°C.

Materials Needed But Not Supplied

- Distilled or deionized water free of urea.
- 96 well plate reader capable of reading optical absorption at 450 nm.
- Software for converting optical density (OD) readings from the plate reader and carrying out quadratic/ rectangular hyperbola fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Urea is a by-product of protein metabolism by the liver, and is therefore removed from the blood by the kidneys. Urea freely filters through the glomerulus, but is reabsorbed by the renal tubules in a flow-dependent fashion. The higher the flow rate, the greater amount of urea nitrogen is cleared from circulation and eliminated through the kidneys. As a result, 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 should be between 7 and 21 mg urea nitrogen per 100 mL blood (mg/dL) (1). Azotemia, poor kidney function, will cause elevated BUN levels ($\geq$ 50 mg/dL) and is associated with acute kidney failure or injury, severe acute pancreatitis, congestive heart failure or gastrointestinal bleeding (2-5). Azotemia also can occur with dehydration, as a result of alcohol abuse, or high protein diets. Lower than expected BUN levels are usually not clinically predictive, but are primarily associated with liver disease or malnutrition, including malabsorption and low protein diets (6). Urine and saliva are considered to be acceptable non-invasive samples for measurement of urea nitrogen (7).

Serum creatinine is another metabolic waste product freely filtered by the glomerulus, but does not undergo tubular reabsorption. Its steady rate of elimination is frequently used to generate an index or ratio with BUN values for normalized evaluations. Creatinine Urinary Detection Kit (SKT-200) and Creatinine Serum Detection Kit (SKT-217) are also available from StressMarq Biosciences.

Assay Overview

The Blood Urea Nitrogen (BUN) Detection Kit is designed to quantitatively measure urea nitrogen in a variety of samples. Please read the complete kit insert before performing this assay. A urea nitrogen standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Samples are mixed with Color Reagents A and B and incubated at room temperature for 30 minutes. The colored product is read at 450 nm. The concentration of urea nitrogen in the sample is calculated, after making a suitable correction for any dilution, using software available with most plate readers. The results are expressed in terms of mg/dL urea nitrogen. If samples are to be expressed in terms of mg/dL urea, the data can be converted using the multiplier 2.14.

The urease enzyme (Catalog No. SKC-213E) is provided as a means of assessing outlier data, and is not essential to the general protocol. There are separate instructions for its use in the "Appendix" section of this booklet.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Serum, Plasma, Urine, Saliva and TCM

Urea nitrogen is identical across all species and this kit will measure urea nitrogen from sources other than human. The end user should evaluate recoveries of urea nitrogen in samples from other species being tested. The kit will measure urea nitrogen in low concentration samples such as RPMI cell culture media, however the media should not contain Phenol Red.

If samples need to be stored after collection, we recommend storing them at -70°C or lower, preferably after being frozen in liquid nitrogen. This assay has been validated for serum, plasma and urine. Samples containing visible particulate should be centrifuged prior to using.

Sample Preparation

NOTE: Please be advised that all suggested dilutions below are simply recommended as a starting point, and it may be necessary to adjust the dilution based on experimental results.

Saliva
Saliva should be clarified by freeze/thawing, followed by centrifugation at 14,000 rpm at 4°C for 10 minutes. The saliva supernatant should be diluted at least 1:2 before measuring in the assay.

Urine
Where concentrations of urea are higher, the recommended final dilution is ≥ 1:100. For highly colored samples, greater dilutions may be necessary.

Serum/ Plasma
For serum or plasma, the recommended dilution is ≥ 1:10 and ≥1:20 respectively.

NOTE: The kit range for this assay is from 0-10 mg/dL BUN. At a 1/10 dilution the high end of normal for blood will be approximately 2.2 mg/dL. Any results higher than this could be subject to further study using the Urease Enzyme (Kit component SKC-213E). Use the protocol in the Appendix if needed:

Standard Preparation

Standard Preparation

Urea Nitrogen Standards are prepared by labeling seven tubes. Briefly vortex to mix. Pipette 360 µL of distilled or deionized water into the first tube and 200 µL into the remaining tubes. Carefully add 40 µL of the Urea Nitrogen Standard to the first tube and vortex completely. Take 200 µL of the solution in the first tube and add it to second tube and vortex completely. Repeat this for the remaining tubes. The concentration of Urea Nitrogen in the tubes is shown below.
Use all Standards within 2 hours of preparation.

	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Blank
Water Volume (µL)	360	200	200	200	200	200	200	400
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	N/A
Volume of Addition (µL)	40	200	200	200	200	200	200	N/A
Final Conc. (mg/dL)	10	5	2.5	1.25	0.625	0.3125	0.156	0

Assay Protocol

Use the plate layout sheet on page 19 to aid in proper sample and standard identification.

1. Pipette 50 µL of samples or appropriate standards into duplicate wells in the plate.
2. Pipette 50 µL of water into duplicate wells as the Zero standard.
3. Add 75 µL of Color Reagent A to each well using a repeater pipette.
4. Add 75 µL of Color Reagent B to each well using a repeater pipette.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density at 450 nm.

ANALYSIS

Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a quadratic or rectangular hyperbola fit, after subtracting the mean OD's for the blank. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

Sample	Mean OD	Net OD	Urea Nitrogen Concentration (mg/dL)
Zero	0.391	0.000	0
Standard 1	2.533	2.143	10
Standard 2	1.813	1.423	5
Standard 3	1.218	0.828	2.5
Standard 4	0.845	0.455	1.25
Standard 5	0.631	0.241	0.625
Standard 6	0.514	0.124	0.3125
Standard 7	0.455	0.064	0.156

Typical Standard Curve

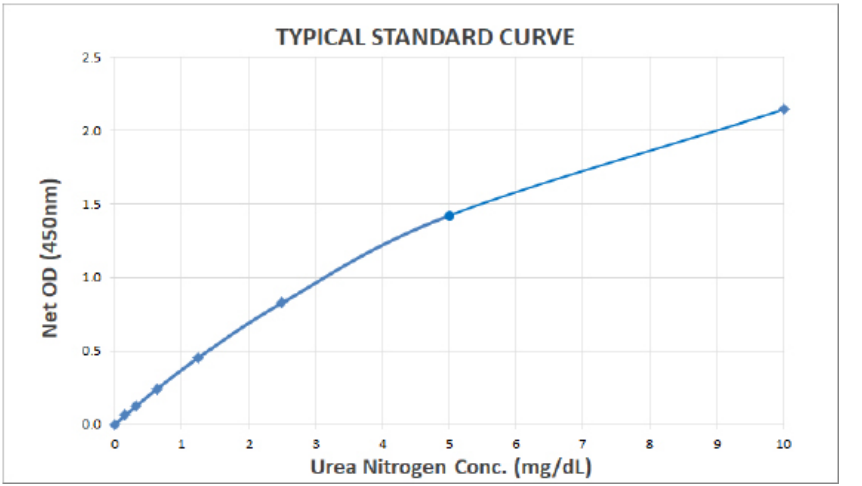


Figure 1. Typical standard curve

NOTE: This typical standard curve was generated using the Blood Urea Nitrogen Detection Kit Protocol. This standard curve is for demonstration only. A standard curve must be generated for each assay.

Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.
Sensitivity was determined as 0.042 mg/dL.

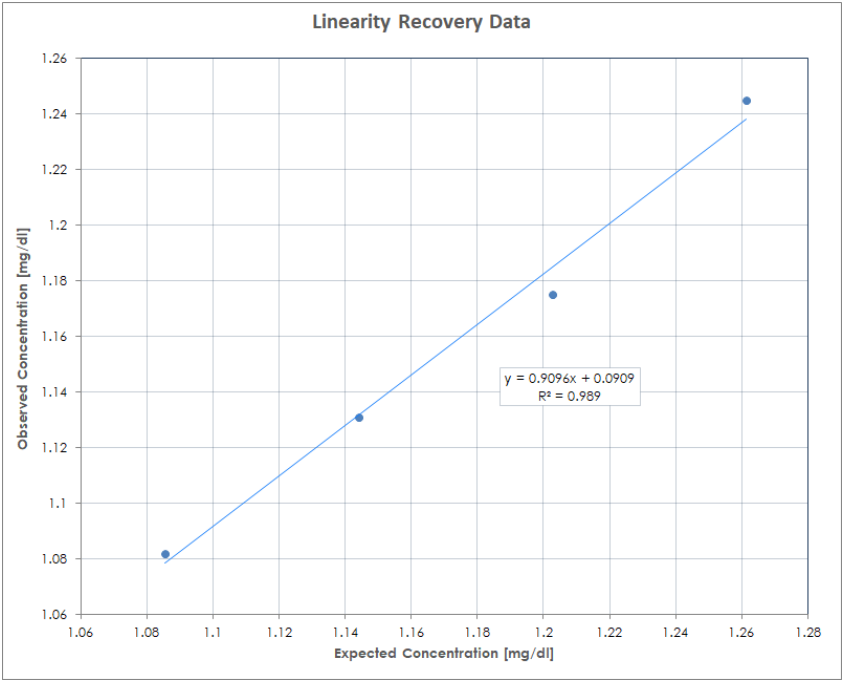
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.
The Limit of Detection was determined as 0.065 mg/dL.

Linearity

Linearity was determined by taking two human serum samples with known BUN concentrations and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High serum	Low Serum	Observed Conc. (mg/dL)	Expected Conc. (mg/dL)	% Recovery
80%	20%	1.25	1.26	98.7
60%	40%	1.18	1.20	97.7
40%	60%	1.13	1.14	98.8
20%	80%	1.08	1.09	99.7
			Mean Recovery	**98.7%**

Figure 2. Linearity Recovery Data


Linearity Recovery Data
Observed Concentration [mg/dl]
Expected Concentration [mg/dl]
y = 0.9096x + 0.0909
R² = 0.989
1.26
1.24
1.22
1.2
1.18
1.16
1.14
1.12
1.1
1.08
1.06
1.06
1.08
1.1
1.12
1.14
1.16
1.18
1.2
1.22
1.24
1.26
1.28

Precision

1. Intra-Assay Precision (Within Run Precision)

- To determine Intra-Assay Precision, three samples of known concentration were assayed at different times, with different operators. The intra-assay coefficient of variation of the BUN within assays was determined to be <10%.

2. Inter-Assay Precision (Between Run Precision)

- To determine Inter-Assay Precision, three samples of known concentration were assayed at different times, with different operators. The inter-assay coefficient of variation of the BUN between assays was determined to be <20%.

Sample Values

Five random adult urines were diluted and tested in this assay. The Urea Nitrogen values widely ranged from 463.4 to 1075.3 mg/dL as expected for random urine sampling.

Interferents

Ammonia (as ammonium hydroxide) at concentrations of 81.9 mM to 81.9 nM were run in the assay. These concentrations gave no optical density in the assay, indicating zero interference from ammonia in the assay.

References

1. Laboratory reference values. Urea nitrogen (BUN). Rochester, Minn.: Mayo Foundation for Medical Education and Research; Nov. 2010.
2. Waiker SS and Bonventre JV. (2008) Biomarkers for the diagnosis of acute kidney injury. Nephron Clin. Pract. 109: c192-c197.
3. Al Mofleh IA. World J. Gastroent. (2008) Severe acute pancreatitis: pathogenetic aspects and prognostic factors. Congestive heart failure. 14(5): 675-684.
4. Iglesiase J. *et al.* (2006) Predictors of worsening renal function in adult patients with heart failure receiving recombinant human B-type brain natriuretic peptide (nesiritide). Nephrol. Dial. Transplant. 21: 3458-3465.
5. Mayo Clinic. Blood urea nitrogen (BUN) tests. http://www.mayoclinic.com/health/blood-urea-nitrogen/MY00373/DSECTION=results
6. Lum G and Leal-Khouri S. (1989) Significance of low serum urea nitrogen concentrations. Clin. Chem. 35(4): 639-640.
7. Akai T, *et al.* (1983) Salivary urea nitrogen as an index to renal function: a test strip method. Clin. Chem. 29(10): 1825-1827.

Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

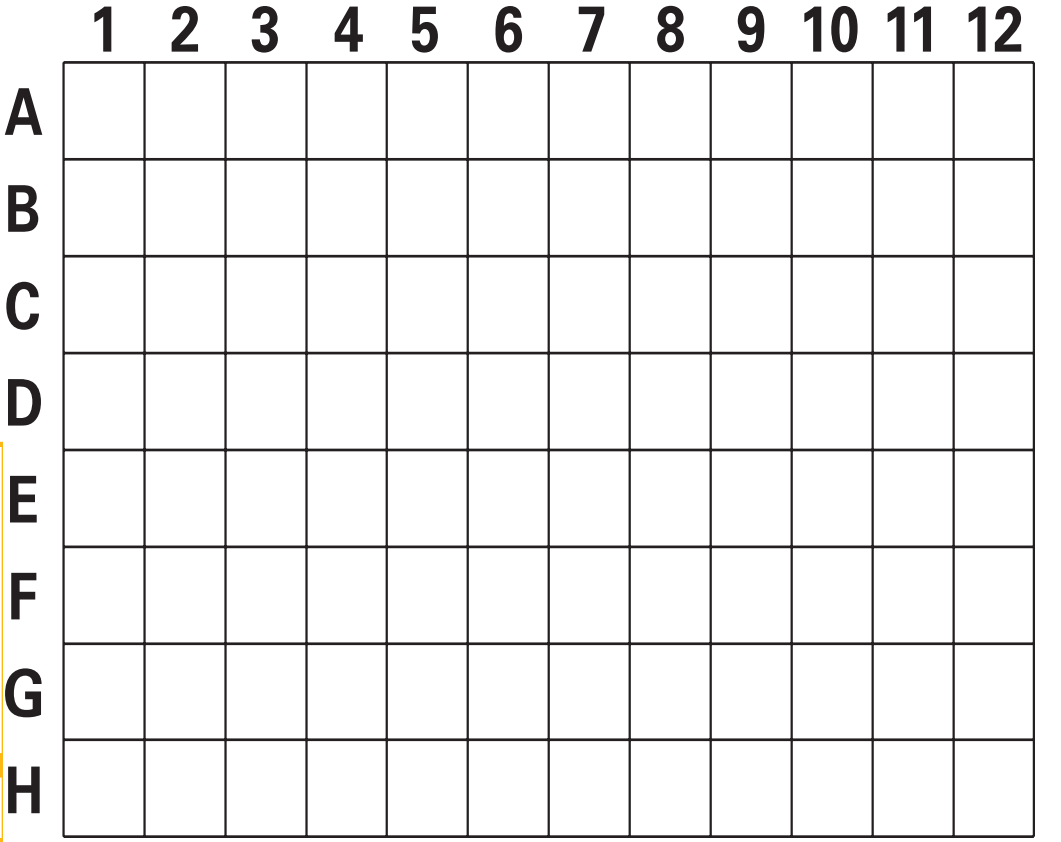
Contact Information

Technical Service Contact Information

Phone: 250-294-9065
Fax: 250-294-9025
E-Mail: techsupport@stressmarq.com
Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

NOTES


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H

APPENDIX

The kit range for this assay is from 0-10 mg/dL BUN. At a 1/10 dilution the high end of normal for blood will be approximately 2.2 mg/dL. Any results higher than this could be subject to further study using the Urease Enzyme (Kit component SKC-213E). Use this protocol if needed (see Figure 3):

1. Each vial of SKC-213E contains urease. Reconstitute the urease with 250 µL of water or pH7.4 phosphate buffer. *Note: The reconstituted urease should be kept on ice, and used within a few hours.*
2. The sample should be diluted to 2 mg/dL. Calculate the amount of water necessary for this dilution, less 1/6 (enzyme spike volume in step 3) of the total final sample volume desired.
3. Determine the enzyme spike volume by multiplying the final volume of the sample by 0.17 (1/6). Add this volume to your diluted sample. Vortex and centrifuge.
4. Urease Activity Positive Control: Calculate and dilute the standard to 2 mg/dL, taking into account the urease enzyme spike volume (1/6 of the final volume). Add 50 µL of urease spiked 2 mg/dL urea into duplicate wells in the plate.
5. Add 50 µL of enzyme spiked diluted sample into duplicate wells in the plate.
6. Cover and incubate at room temperature for 30 minutes.
7. Create a duplicate standard curve as described previously, alongside the samples and the controls.
8. Add 75 µL of Color Reagent A to each well.
9. Add 75 µL of Color Reagent B to each well.
10. Incubate at room temperature for 30 minutes.
11. Read the optical density at 450 nm.

After treatment with urease, the disappearance of the signal indicates the presence of urea. The retention of the signal may indicate positive interference.

	Urease Positive Control	Sample
Water Volume (µL)	76	*TBD using the following equation: [100µL - Volume of Addition µL]
Addition	Standard 1 (10 mg/dL)	Sample
Volume of Addition (µL)	24	*TBD using the following equation: $\left[\frac{2\ \text{mg/dL}}{\text{Concentration of Sample mg/dL}}\right]$ *(120µL)
Final Concentration (mg/dL)	2	2
Volume of Urease (µL)	20	20
Final Volume (µL)	120	120

Figure 3. BUN Urease Activity Positive Control & Urease Spiked Sample

Please also note that the following are inhibitors of urease, so attention may need to be given to sample preparations:

- 2-mercaptoethanol
- acetohydroxamate
- EDTA
- phosphoramidate
- fluoride ion
- 1,4-benzoquinone
- 2,5-dimethyl-1,4-benzoquinone

EXAMPLE:

Two Human serum samples, diluted 1/10, were determined to contain 10mg/dL BUN. The normal expected value for a 1/10 diluted Human serum sample is 0.7 to 2.0 mg/dL. Therefore, the samples were considered outliers (outside of the normal range). Since it is possible that a substance which was not urea may have led to false positive interference, urease sample treatment was necessary to confirm that the determination was derived from blood urea nitrogen alone. The Human serum samples were diluted to 2mg/mL and spiked 1/6th volume with reconstituted urease enzyme, incubated for 30min at RT as described in the manual addendum for the urease treatment protocol.

Results (refer to Figure 4, below): Human serum sample 01 was determined to contain -0.02 mg/dL BUN after treatment with urease, but the sample02 determination was unchanged even after an identical treatment with urease.

Conclusions: the determination of urea in sample01 has been validated via the urease enzyme test, however the determination of urea in sample02 may have been due to contamination via positive interference (or the sample may be contaminated with a urease enzyme inhibitor).

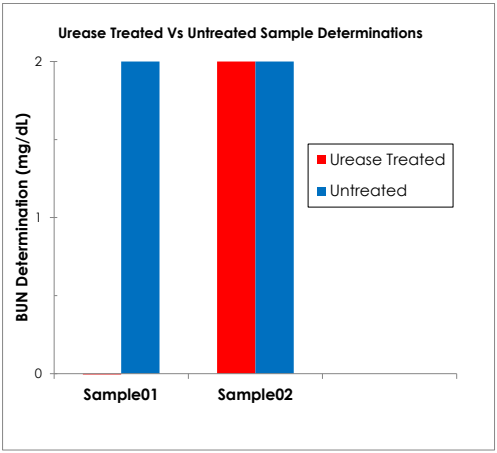


Figure 4.Example results showing urease treated vs untreated sample determinations