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**StressXpress®**

## **Blood Urea Nitrogen Detection Kit**

Catalog# SKT-213 (2 Plate kit)

Quantitative colorimetric measurement of urea nitrogen



## TABLE OF CONTENTS

<b>GENERAL INFORMATION</b>	4	<b>Materials Supplied</b>
	5	<b>Precautions</b>
	5	<b>Storage</b>
	5	<b>Materials Needed but Not Supplied</b>
<b>INTRODUCTION</b>	6	<b>Background</b>
	7	<b>Assay Overview</b>
<b>PRE-ASSAY PREPARATION</b>	8	<b>Sample Types</b>
	8	<b>Sample Preparation</b>
	9	<b>Standard Preparation</b>
<b>ASSAY PROTOCOL</b>	10	<b>Assay Protocol</b>
<b>ANALYSIS</b>	11	<b>Calculation of Results</b>
	11	<b>Typical Data</b>
	13	<b>Validation Data</b>
	15	<b>Sample Values</b>
	15	<b>Interferents</b>
<b>RESOURCES</b>	16	<b>References</b>
	17	<b>Warranty and Contact Information</b>
	18	<b>Plate Template</b>
	19	<b>Notes</b>

## GENERAL INFORMATION

### Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-213A	Clear 96 well Plates	2 plates	-
SKC-213B	Urea Nitrogen Standard Calibrated to NIST Standard Reference Material Lot Number 912a	250 $\mu$ L	Urea Nitrogen at 100 mg/dL in a special stabilizing solution
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

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 components of this kit should be stored at room temperature until the expiration date of the kit.

## 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 four parameter logistic curve (4PLC) 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.

### 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 Urea Nitrogen (BUN) Colorimetric 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 calibrated to NIST reference materials 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.

## 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  $-70^{\circ}\text{C}$  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

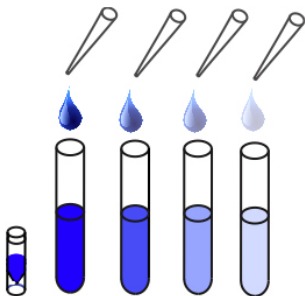
Dilute sample with distilled or deionized water prior to running in the assay. For serum or plasma, the recommended dilution is  $\geq 1:10$  and  $\geq 1:20$  respectively. Saliva should be clarified by freeze/thawing, followed by centrifugation at 14,000 rpm at  $4^{\circ}\text{C}$  for 10 minutes. The saliva supernatant should be diluted at least 1:2 before measuring in the assay. For urine, where concentrations of urea are higher, the recommended final dilution is  $\geq 1:100$ . For highly colored samples, dilution greater than 1:10 or 1:100 may be necessary.



## Standard Preparation

### Standard Preparation

Urea Nitrogen Standards are prepared by labeling seven tubes. Briefly vortex to mix. Pipet 360  $\mu\text{L}$  of distilled or deionized water into the first tube and 200  $\mu\text{L}$  into the remaining tubes. Carefully add 40  $\mu\text{L}$  of the Urea Nitrogen Standard to the first tube and vortex completely. Take 200  $\mu\text{L}$  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
Water Volume ( $\mu\text{L}$ )	360	200	200	200	200	200	200
Addition	Stock	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6
Volume of Addition ( $\mu\text{L}$ )	40	200	200	200	200	200	200
Final Concentration (mg/dL)	10	5	2.5	1.25	0.625	0.3125	0.156

### Assay Protocol

Use the plate layout sheet on page 16 to aid in proper sample and standard identification.

1. Pipet 50  $\mu\text{L}$  of samples or appropriate standards into duplicate wells in the plate.
2. Pipet 50  $\mu\text{L}$  of water into duplicate wells as the Zero standard.
3. Add 75  $\mu\text{L}$  of Color Reagent A to each well using a repeater pipet.
4. Add 75  $\mu\text{L}$  of Color Reagent B to each well using a repeater pipet.
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 four-parameter logistic curve (4PLC) 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.

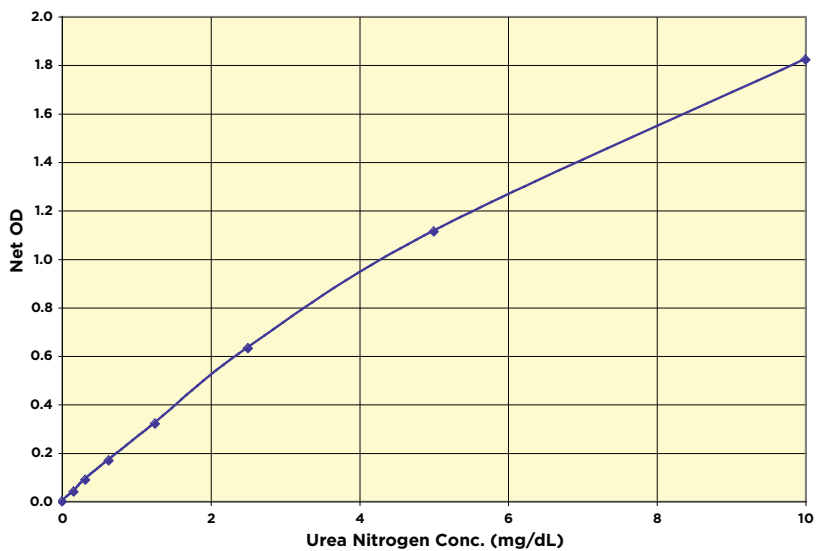
### Typical Data

Sample	Mean OD	Net OD	Urea Nitrogen Concentration (mg/dL)
Zero	0.361	0	0
Standard 1	2.184	1.823	10
Standard 2	1.474	1.113	5
Standard 3	0.993	0.632	2.5
Standard 4	0.682	0.321	1.25
Standard 5	0.530	0.169	0.625
Standard 6	0.450	0.089	0.3125
Standard 7	0.401	0.040	0.156
Sample 1	0.686	0.325	1.24
Sample 2	1.451	1.090	4.86

Always run your own standard curves for calculation of results.

Do not use these data.

## Typical Standard Curve



## 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 0.030 mg/dL.

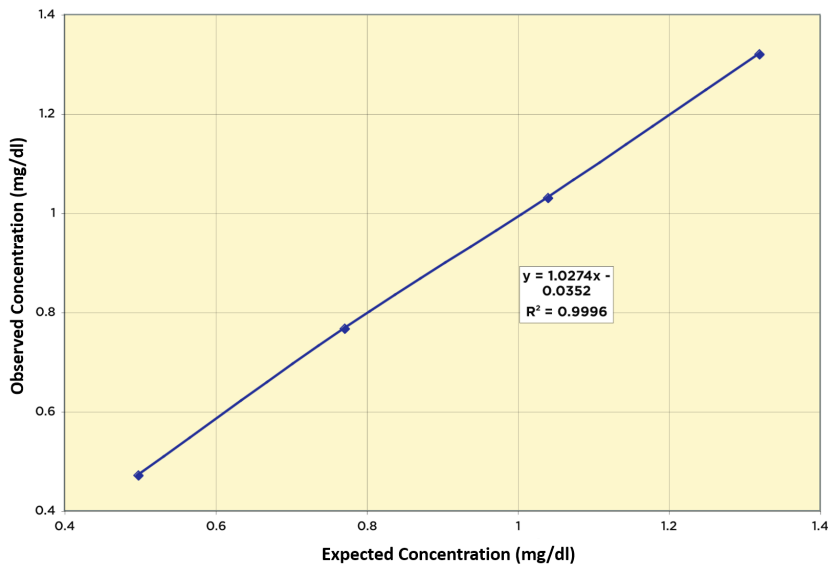
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.

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%	1.32	1.32	99.9
60%	40%	1.03	1.04	98.5
40%	60%	0.767	0.771	99.5
20%	80%	0.471	0.498	94.7
Mean Recovery				98.1%



### Intra Assay Precision

Three human samples were further diluted in water and run in replicates of 20 in an assay. The mean and precision of the calculated concentration were:

Sample	BUN Concentration (mg/dL)	%CV
1	1.24	2.0
2	2.29	1.9
3	4.86	2.8

### Inter Assay Precision

Three human samples were further diluted in water and run in duplicates in twenty-eight assays run over multiple days by five operators. The mean and precision of the calculated concentrations were:

### Sample Values

Six random adult human serum and plasma samples were diluted and tested in the assay. The serum samples ranged from 15.6 to 22.3 mg/dL with an average of 18.6 mg/dL BUN while EDTA and heparin plasma samples ranged from 13.6 to 23.7 mg/dL with an average BUN of 18.1 mg/dL. Six random saliva samples were clarified, diluted and tested in the kit. The Urea Nitrogen values ranged from 4.3 to 11.9 mg/dL, with an average concentration of 8.7 mg/dL. Six random urines were also diluted and tested in the kit. The Urea Nitrogen values widely ranged from 37.2 to 1007.2 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.

## RESOURCES

### References

1. Laboratory reference values. Urea nitrogen (BUN). Rochester, Minn.: Mayo Foundation for Medical Education and Research; Nov. 2010.
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3. Al Mofleh, IA. *World J. Gastroent.* "Severe acute pancreatitis: pathogenetic aspects and prognostic factors." 2008. *Congestive heart failure.* 14(5):675-684.
4. Iglesias, J. et al. "Predictors of worsening renal function in adult patients with heart failure receiving recombinant human B-type brain natriuretic peptide (nesiritide)." *Nephrol. Dial. Transplant.* 2006. 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 S Leal-Khoury. "Significance of low serum urea nitrogen concentrations." *Clin. Chem.* 1989. 35(4):639-640.
7. Akai, T, et al. "Salivary urea nitrogen as an index to renal function: a test strip method". *Clin. Chem.* 1983. 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).



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