Blood Urea Nitrogen (BUN)

Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44114-96

www.zellx.de

Sample Types Validated for:

Serum, Plasma, Urine, Saliva, and Tissue Culture Medium

!!! Caution: This product is for Research Use Only. Not for in vitro Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

Introduction

Background

Urea is a by-product of protein metabolism in the liver, which is removed from the blood circulation by kidneys. Urea is freely filtered through the glomerulous but is reabsorbed by the renal tubules in a flow-dependent fashion. The higher the flow rate, the greater the amount of Urea Nitrogen clearance from circulation and elimination through the kidneys.

As a result, the level of circulating Blood Urea Nitrogen (BUN) serves as a primary measure of kidney function. Normal adult BUN levels should be between 7 and 21 mg per 100 mL blood (mg/dL). Elevated BUN level (≥ 50 mg/dL) is associated with acute kidney failure or injury, severe acute pancreatitis, congestive heart failure or gastrointestinal bleeding. Azotemia is a medical condition caused by the increased level of nitrogen-containing compounds, including BUN and Creatinine, in the blood, as a result of from poor kidney function, dehydration due to 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. Urine and saliva are considered to be the acceptable non-invasive samples for measurement of Urea Nitrogen.

Serum creatinine is another metabolic waste product freely filtered by the glumerulous, 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. Our easy to use Serum creatinine assay (Cat NO. ZX-44111-96) and Urine Creatinine Assay (Cat NO. ZX-44110-96) can be used to measure creatinine level in serum and urine respectively.

Assay principle

The ZellX® BUN assay is designed to quantitatively measure Urea Nitrogen in a variety of samples including Serum, Plasma, Urine, Saliva and Tissue Culture Media. Please read the complete kit insert prior performing the 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 results are expressed in terms of mg/L Urea Nitrogen. If samples are to be expressed in terms of mg/L urea, the data can be converted to mg/dL by dividing to 10 or mg/mL by dividing to 1000.

This kit uses the Urea Nitrogen Standard solutions calibrated to the US National Institute for Science and Technology Standard Reference Materials and ISO/IEC standards.

General information

Materials supplied in the Kit

Component	*Quantity*
Urea Nitrogen Standard (1000 mg/L)	100 µL
Reagent A	7.5 mL
Reagent B	7.5 mL
Clear 96-well Plate	1 plate

Storage instruction

All reagents should be stored at room temperature until the expiration date of the kit.

Materials required but not supplied

Deionized water (diH_2O) free of urea

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Centrifuge, Vortex mixer

Precision pipettes, multichannel/repeater pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

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

General remarks

- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.

- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Sample preparation

Since Urea Nitrogen is identical across all species, it is expected that this kit can measure Urea Nitrogen in human and other species**.**

The kit will measure Urea Nitrogen in low concentration samples such as RPMI cell culture media, however the media should not contain Phenol Red.

Samples containing visible particulate should be centrifuged prior to conducting the assay.

All samples and standards must be used within 2 hours of preparation or must be stored at ≤ -70, preferably after being frozen in liquid nitrogen for later analysis.

I. **Serum, Plasma:**
- Collect the fresh serum or plasma with heparin or EDTA.
- Centrifuge at 600 g for 10 min at 4˚C.
- Separate the serum or plasma from the red blood cells, and transfer into fresh tubes.
- Serum should be diluted ≥ 1:10 and plasma ≥ 1:20 with diH_2O. In case of serum, take one volume of sample and add 9 or more volumes of diH_2O, and in case of plasma, mix one volume of sample with 19 or more volumes of diH_2O.

II. **Urine:**
- Urine should be diluted ≥ 1:100 by taking one part of sample and adding 99 or more parts of diH_2O prior to conducting assay. Due to the levels of urea found in urine, dilutions may need to be > 1:100.
- **Normalize the sample value based on creatinine levels using our Urine Creatinine assay kit Cat NO. ZX-44110-96 in random urine specimen.**

III. **Saliva:**
- Saliva samples should be frozen and thawed, then centrifuged at 14,000 rpm for 10 minutes.
- Supernatant should be diluted ≥ 1:2 by taking one part of sample and adding 1 or more parts of diH_2O prior to conducting the assay

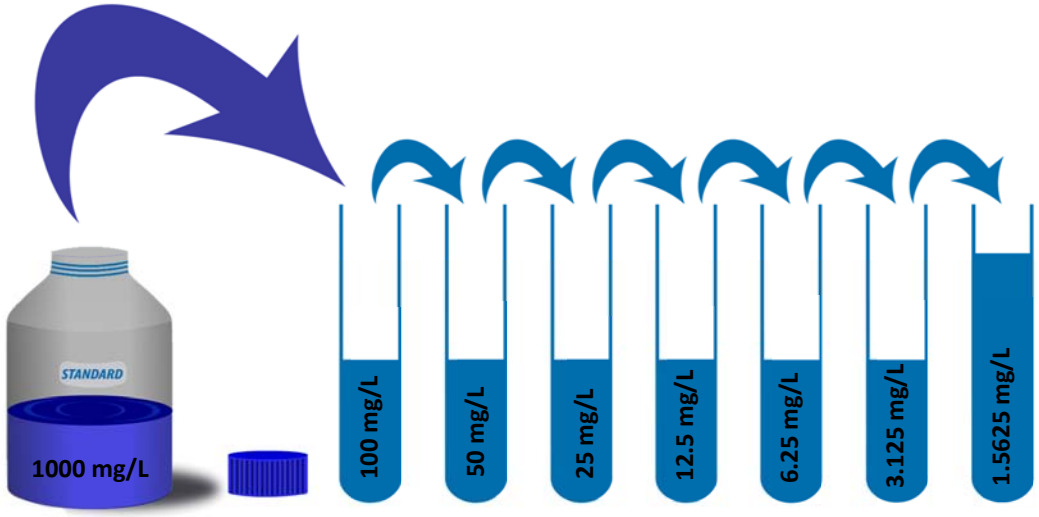
All the samples must be used within 2 hours of preparation; otherwise, aliquots of the sample should be kept at ≤ -70˚C, preferably after being frozen in liquid nitrogen for later use.

Standard preparation

Standard solutions:

- Prepare a 1:10 dilution of Standard with diH_2O (mix 40 µL of Urea Nitrogen standard with 360 µL of diH_2O), and label as the Standard No.7 (100 mg/L).
- Apply series of other dilutions as described in the table.
- The diH_2O is used as the 0 mg/L standard.

No.	*Concentration Nitrite or Nitrate*	*Material needed*
Standard No.7	100 mg/L	40 µL Standard (1000 mg/L) + 360 µL diH_2O
Standard No.6	50 mg/L	200 µL Standard No.7 + 200 µL diH_2O
Standard No.5	25 mg/L	200 µL Standard No.6 + 200 µL diH_2O
Standard No.4	12.5 mg/L	200 µL Standard No.5 + 200 µL diH_2O
Standard No.3	6.25 mg/L	200 µL Standard No.4 + 200 µL diH_2O
Standard No.2	3.125 mg/L	200 µL Standard No.3 + 200 µL diH_2O
Standard No.1	1.5625 mg/L	200 µL Standard No.2 + 200 µL diH_2O
Standard No.0	0 mg/L	200 µL diH_2O



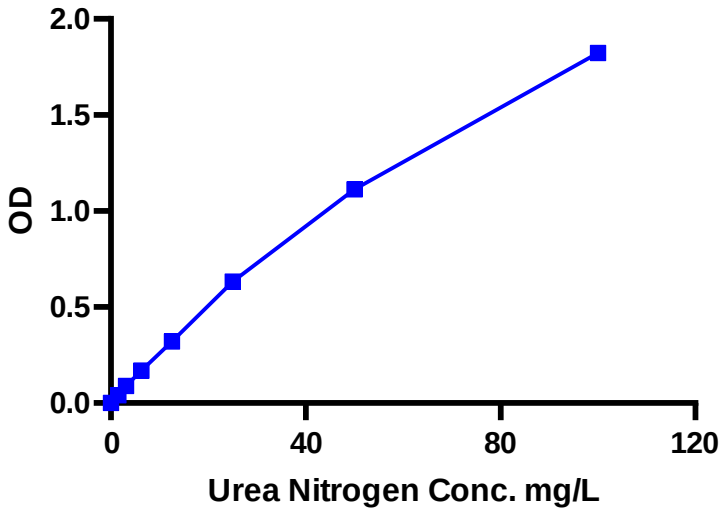
All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipette 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 50 µL of diH_2O into duplicate wells as the Zero standard.
3. Add 75 µL of the Reagent A to each well using a multichannel/repeater pipette.
4. Add 75 µL of the Reagent B to each well using a multichannel/repeater pipette.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs for the zero standard from all OD values
  (for example if the OD value of zero standard, and standard 7 are 0.087, and 1.086 respectively; then the adjusted ODs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.



A typical standard curve of ZellX® BUN Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX® BUN assay was determined as 0.65 mg/L.

Sensitivity

The sensitivity of the ZellX® BUN assay was determined as 0.3 mg/L.

Precision

Intra-Assay Precision (Precision within an assay): 3 human samples were tested 20 times in an assay.

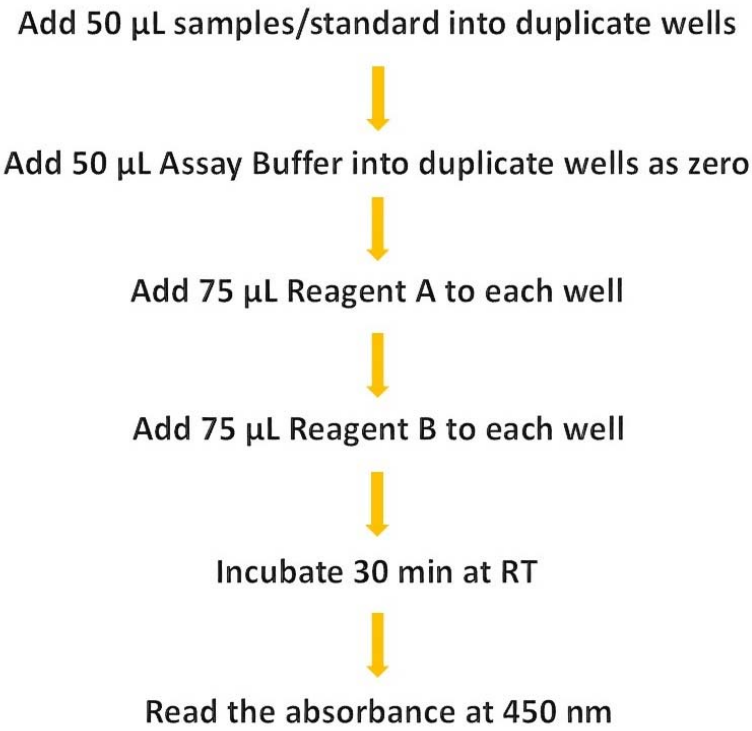
Inter-Assay Precision (Precision between assays): 3 human samples were tested in duplicate on 28 different assays over multiple days.

Item	*%CV*
Intra assay	2.8, 1.9, 2.0
Inter assay	3.1, 3.3, 4.3

Interferences

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.

Protocol summary



References

1. Laboratory reference values. Urea Nitrogen (BUN). Rochester, Minn.: Mayo Foundation for Medical Education and Research; Nov. 2010.
2. Waiker, SS and JV Bonventre. “Biomarkers for the diagnosis of acute kidney injury.” Nephron Clin. Pract. 2008. 109:c192-c197.
3. Al Mofleh, IA. World J. Gastroent. “Severe acute pancreatitis: pathogenetic aspects and prognostic factors.” 2008. Congestive heart failure. 14(5):675-684.
4. Iglesiase, J. et al. “Predictors of worsening renal function in adult patients with heart failure receiving recombinant human B-type brain natruiretic peptide (nesiritide).” Nephrol. Dial. Transplant. 2006. 21:3458-3465.
5. Mayo Clinic. “Blood urea nitrogen (BUN) tests.” www.mayoclinic.com/health/blood-urea-nitrogen/MY00373/DSECTION=results
6. Lum, G and S Leal-Khouri. “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.

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