KAMIYA BIOMEDICAL COMPANY

Urea Nitrogen (BUN) detection Kit

For the quantitative determination of urea nitrogen in saliva and TCM

Cat. No. KT-747

For Research Use Only.

PRODUCT INFORMATION

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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 glomerulous, 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). Azotemia, poor kidney function, will cause elevated BUN levels (≥ 50 mg/dL) and is associated with acute kidney failure or injury, severe acute pancreatitis, congestive heart failure or gastrointestinal bleeding. 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. Urine and saliva are considered to be 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.

PRINCIPLE

The Urea Nitrogen (also called 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 calibrator calibrated to NIST reference materials is provided to generate a calibration curve for the assay and all samples should be read off the calibration 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.

COMPONENTS

Clear 96 well Plates Bags containing 96 well plates
2 plates

Urea Nitrogen Calibrator Urea Nitrogen at 100 mg/dL in a special stabilizing solution.
250 µL
Calibrated to NIST Standard Reference Material Lot Number 912a

Color Reagent A An acidic solution of Color Reagent A. **CAUTION: CAUSTIC**
15 mL

Color Reagent B An acidic solution of Color Reagent B. **CAUTION: CAUSTIC**
15 mL

STORAGE

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

OTHER MATERIALS REQUIRED

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.

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.

SAMPLE TYPES

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

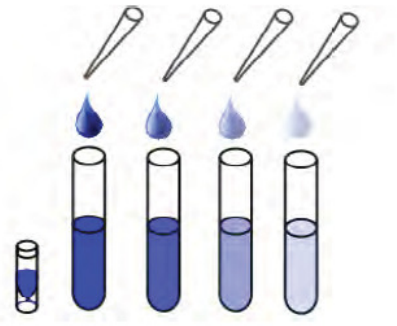
Dilute sample with distilled or deionized water prior to running in the assay. For serum or plasma, the recommended dilution is ≥ 1:10 and ≥1:20 respectively. Saliva should be clarified by freeze/thawing, followed by centrifugation at 14,000 rpm at 4°C for 10 minutes. The saliva supernatant should be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

CALIBRATOR PREPARATION

Calibrator Preparation

Urea Nitrogen Calibrators are prepared by labeling seven tubes. Briefly vortex to mix. Pipet 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 Calibrator 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 Calibrators within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Water Vol (µL)	**360**	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol of Addition (µL)	**40**	200	200	200	200	200	200
Final Conc (mg/dL)	10	5	2.5	1.25	0.625	0.3125	0.156

ASSAY PROTOCOL

Use the plate layout sheet on the back page to aid in proper sample and calibrator identification.

1. Pipet 50 µL of samples or appropriate calibrators into duplicate wells in the plate.

3. Pipet 50 µL of water into duplicate wells as the Zero calibrator.

4. Add 75 µL of Color Reagent A to each well using a repeater pipet.

5. Add 75 µL of Color Reagent B to each well using a repeater pipet.

6. Incubate at room temperature for 30 minutes.

7. Read the optical density at 450 nm.

CALCULATION OF RESULTS

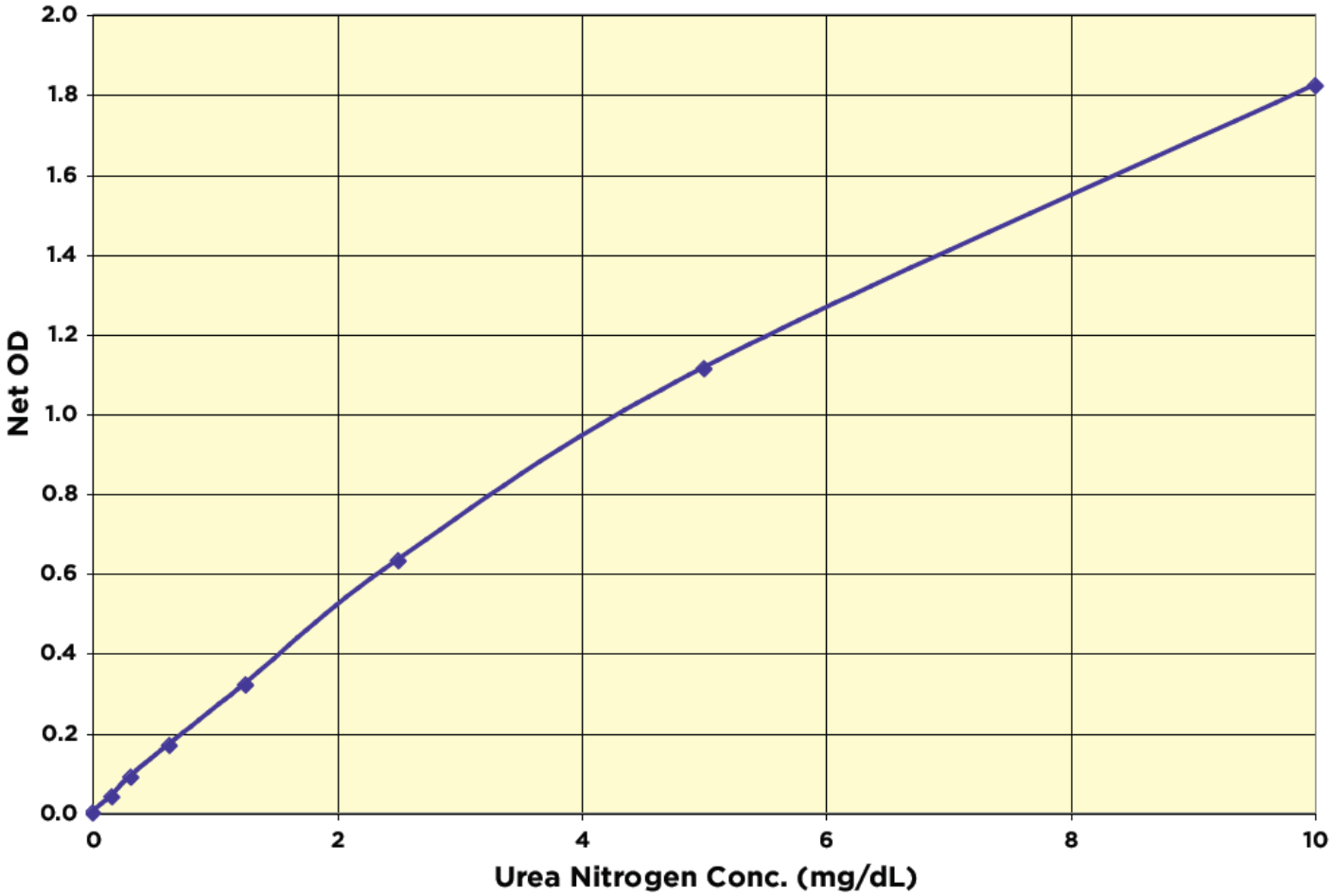
Average the duplicate OD readings for each calibrator and sample. Create a calibration curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit, after subtracting the mean OD's for the blank. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL DATA

Sample	Mean OD	Net OD	Urea Nitrogen Conc. (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 calibration curves for calculation of results. Do not use these data.

Typical Calibration Curve



Always run your own calibration curves for calculation of results. Do not use these data.

VALIDATION DATA

Sensitivity and Limit of Detection

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

Sensitivity was determined as 0.030 mg/dL.

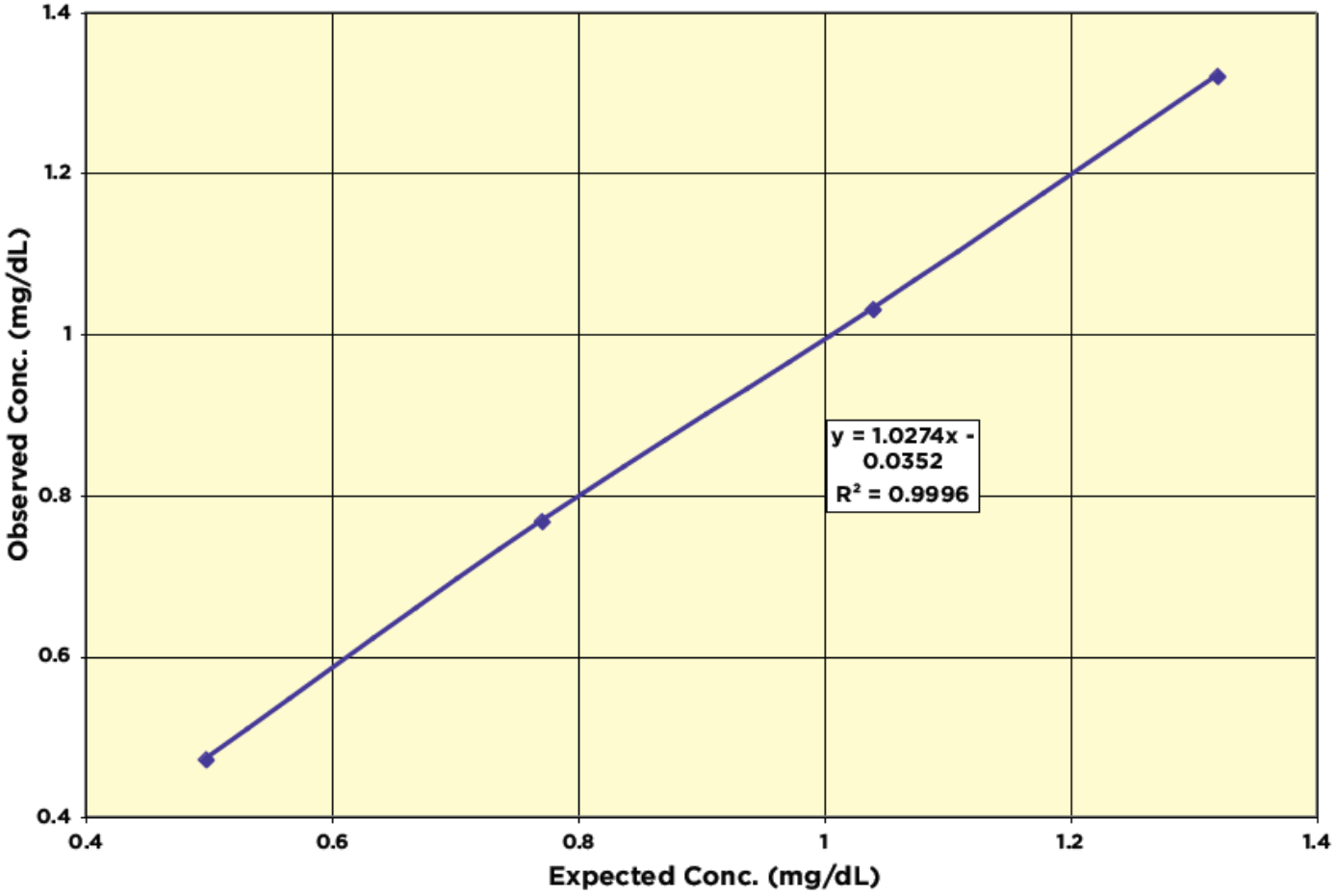
The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration human sample.

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 concentrations were:

Sample	BUN Conc. (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	BUN Conc. (mg/dL)	%CV
1	1.29	3.1
2	2.35	4.3
3	5.18	3.3

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.

FOR RESEARCH USE ONLY

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