Urea Nitrogen (BUN) Colorimetric Detection Kit

Catalog Number EIABUN (192 tests)

Pub. No. MAN0025406 **Rev A**.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Urea Nitrogen (BUN) Colorimetric Detection Kit is designed to detect and quantify the level of urea in serum, plasma, urine, saliva, or tissue culture medium. The assay was characterized with human samples, but can be used to test samples from other species.

Urea is a metabolic by-product removed from the blood by the kidneys. 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 range between 7 and 21 mg urea nitrogen per 100 mL blood (mg/dL).

Serum creatinine is another metabolic waste product removed from the blood by the kidneys, but it exhibits a steady rate of elimination, and can be used to normalize BUN values.

Contents and storage

Kit and components are shipped at 4°C. Upon receipt, store the kit at 4°C. Once open, use within 2 weeks.

Components	Quantity
Urea Nitrogen Standard; 100 mg/dL urea nitrogen in a special stabilizing solution	250 µL
Clear 96-well Plate	2 plates
Color Reagent A; acidic solution, **CAUSITC**	15 mL
Color Reagent B; acidic solution, **CAUSITC**	15 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- Freeze-thaw **saliva** samples and centrifuge at 14,000 rpm for 10 minutes at 4°C to clarify sample.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- The assay is compatible with low concentration samples in tissue culture media **not** containing phenol red.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

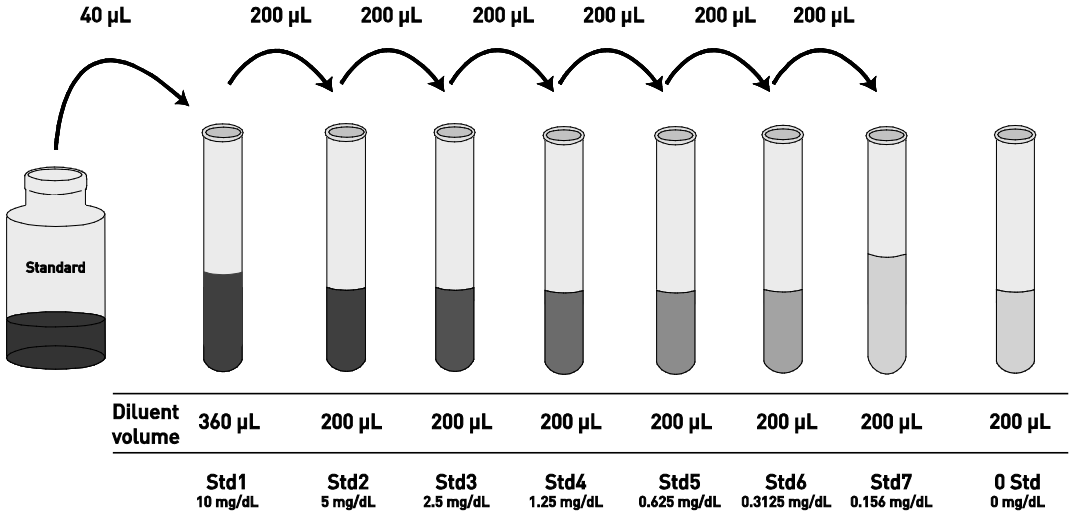
- Dilute **serum** samples ≥1:10 in distilled or deionized water.
- Dilute **plasma** samples ≥1:20 in distilled or deionized water.
- Dilute **clarified saliva** samples ≥1:2 in distilled or deionized water.
- Dilute **urine** samples ≥1:100 in distilled or deionized water.
- For samples that are highly colored, dilutions of ≥1:100 may be necessary.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Urea Nitrogen Standard is calibrated to NIST Standard Reference Material Lot No. 912b.

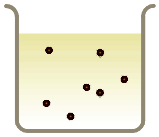
1. Add 40 µL Urea Nitrogen Standard to one tube containing 360 µL distilled or deionized water and label as 10 mg/dL BUN.
2. Add 200 µL distilled or deionized water to each of 7 tubes labeled as follows: 5, 2.5, 1.25, 0.625, 0.3215, 0.156, and 0 mg/dL BUN.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Assay procedure

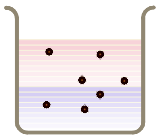
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add color reagent

1. Add 75 µL Color Reagent A into each well.
2. Add 75 µL Color Reagent B into each well.
3. Incubate for 30 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in distilled or deionized water and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–10 mg/dL urea nitrogen.

Standard Urea Nitrogen (mg/dL)	Optical Density (450 nm)
10	2.184
5	1.474
2.5	0.993
1.25	0.682
0.625	0.530
0.3125	0.450
0.156	0.401
0	0.361

Intra-assay precision

Three human samples were assayed in replicates of 20 to determine precision within an assay.

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

CV = Coefficient of Variation

Inter-assay precision

Three human samples were assayed 28 times in duplicate by five operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/dL)	1.29	2.35	5.18
%CV	3.1	4.3	3.3

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Random samples were evaluated for the presence of BUN in this assay.

Sample	Range (mg/dL)	Average (mg/dL)
Serum	15.6–22.3	18.6
Plasma (EDTA and heparin)	13.6–23.7	18.1
Saliva	4.3–11.9	8.7
Urine	37.2–1007.2	—

Interferents

Samples of 81.9 mM to 81.9 nM ammonium hydroxide were tested with the kit. No optical density was measured, indicating zero interference from ammonia in the assay.

Linearity of dilution

Linearity was determined by assaying high and low concentration BUN samples mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (mg/dL)	Observed Conc. (mg/dL)	% Recovery
80	20	1.32	1.32	99.9
60	40	1.04	1.03	98.5
40	60	0.771	0.767	99.5
20	80	0.498	0.471	94.7
			Mean Recovery	**98.1%**

Sensitivity

The analytical sensitivity of the assay is 0.30 mg/dL urea nitrogen. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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