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BIOO SCIENTIFIC

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MAX SIGNAL®

Urea Enzymatic Assay Kit Manual

Catalog #: 1051

Reference #: 1051-01

TABLE OF CONTENTS

GENERAL INFORMATION

Product Description

The *MaxSignal® Urea Enzymatic Assay Kit* is a colorimetric assay for the determination of urea in liquid samples.

MaxSignal® Urea Enzymatic Assay Kit uses an enzyme-based assay to determine urea in milk and other liquid samples. The kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations, to determine urea levels in dairy products in response to customer concerns about food safety. The test is based on a highly proven method for urea determination. The *MaxSignal® Urea Enzymatic Assay Kit* contains sufficient materials to test 21 samples in duplicate.

The assay utilizes urease, a metabolic enzyme, to specifically detect urea in fluids. The *MaxSignal® Urea Enzymatic Assay Kit* provides rapid, accurate, proven results even in complex liquid mixtures. The limit of detection for the test is 40 ppm urea for milk. The linear range of the assay is 40 – 1200 ppm analyte.

The unique features of the kit are:

- Rapid and simple method.
- Minimal sample prep.
- Highly accurate and reproducible.

The kit is designed to be used with a microplate reader. The kit contains urea standards to construct a linear calibration curve and verify assay performance.

Procedure Overview

The *MaxSignal® Urea Enzymatic Assay Kit* measures the concentration of urea using the urease enzyme, which converts urea to ammonia.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} CO_2 + 2\,NH_3$$

The ammonia produced from the urea is then directly detected by a colorimetric chemical reaction.

Kit Contents, Storage and Shelf Life

The *MaxSignal® Urea Enzymatic Assay Kit* has the capacity for 96 determinations or testing of 21 samples in duplicate (using 12 wells for standards). Store the kit at 4°C. The shelf life is 4 months when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	Room temp
Urea Standards:		
0 ppm (white cap tube)	1.6 mL	
8 ppm (yellow cap tube)	1.6 mL	
24 ppm (orange cap tube)	1.6 mL	4°C
60 ppm (pink cap tube)	1.6 mL	
120 ppm (purple cap tube)	1.6 mL	
240 ppm (blue cap tube)	1.6 mL	
Urease Solution	2 x 1.5 ml (3 ml total)	4°C
Phenol Prusside	2 x 1.5 ml (3 ml total)	4°C
6X Alkaline Hypochlorite	2 x 1.5 ml (3 ml total)	4°C

Required Materials/Equipment Not Provided With the Kit

- Microtiter plate reader (*with 590 nm absorbance filter*)
- Microcentrifuge
- Microcentrifuge tubes
- Multichannel pipet (*recommended*)

Warnings and Precautions

BIOO strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport@biooscientific.com.

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

SAMPLE PREPARATION

Milk and Other Liquid Dairy Samples

1. Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days.
2. Transfer 1 ml of each milk sample to a microcentrifuge tube. Centrifuge 10 min at 5000 rpm. The milk fat will form a layer at the top of the sample.
3. Transfer 100 µL of the *lower* aqueous layer to a clean tube (avoid transferring the top fat layer). Add 400 µL of dH_2O to each sample.

Note: Dilution factor: 5

Serum

1. Allow 0.2 – 1 mL blood sample to coagulate in a microfuge tube for 20 min at 37°C and then centrifuge for 5 in at 9,000 rpm.
2. Transfer the supernatant (serum) to clean tube. It is best to test the serum immediately; however if the sample is not be tested right away (within 6 hours of collection), store the serum samples at 4°C and test no later than 3 days after collection.

Note: Dilution factor: 1

UREA DETERMINATION TEST PROTOCOL

Set-Up

1. Warm up kit reagents to room temperature. Turn on instrument (plate reader), allow the light source to warm up, and set absorbance wavelength to 590 nm.
2. Dilute 3 ml of 6X Alkaline Hypochlorite with 15 ml dH_2O to make the 1X reagent (enough for more than 96 reactions). If only part of the kit (< 96 samples) is to be used, dilute the appropriate volume of 6X Alkaline Hypochlorite (30 µL per well) with 5 volumes dH_2O (150 µL per well). Store the remaining portion of 6X Alkaline Hypochlorite at 4 °C.

Test Procedure

Note: Samples such as milk often contain significant levels of ammonia and urea. By testing each sample in duplicate with and without the urease enzyme, the signal arising from endogenous ammonia can be easily distinguished from the urea signal as described below.

1. Liquid dairy samples: Transfer 5 µL of each diluted sample to four wells (in quadruplicate) of the microtiter plate; then add 30 µL of urease solution to two of the wells and 30 µL water to the other two wells (the difference in signal between these two pairs is the urea-specific signal). Tap plate gently 3-4 times to mix sample and enzyme. Incubate 20 min at room temperature.
   Serum: Add 5 µL of serum (in quadruplicate) to four microplate wells; then add 30 µL of urease solution to two of the wells and 30 µL water to the other two wells (the difference in signal between these two pairs is the urea-specific signal). Tap plate gently 3-4 times to mix sample and enzyme. Incubate 20 min at room temperature.
2. Add 30 µL of phenol nitroprusside and 180 µL 1X Alkaline Hypochlorite to each well. Incubate for 20 min at room temperature.
3. Measure the absorbance of each sample in duplicate at 590 nm.

Standard Curve Construction

A calibration curve constructed using the urea standards supplied with the kit is required to determine the urea concentration in the samples.

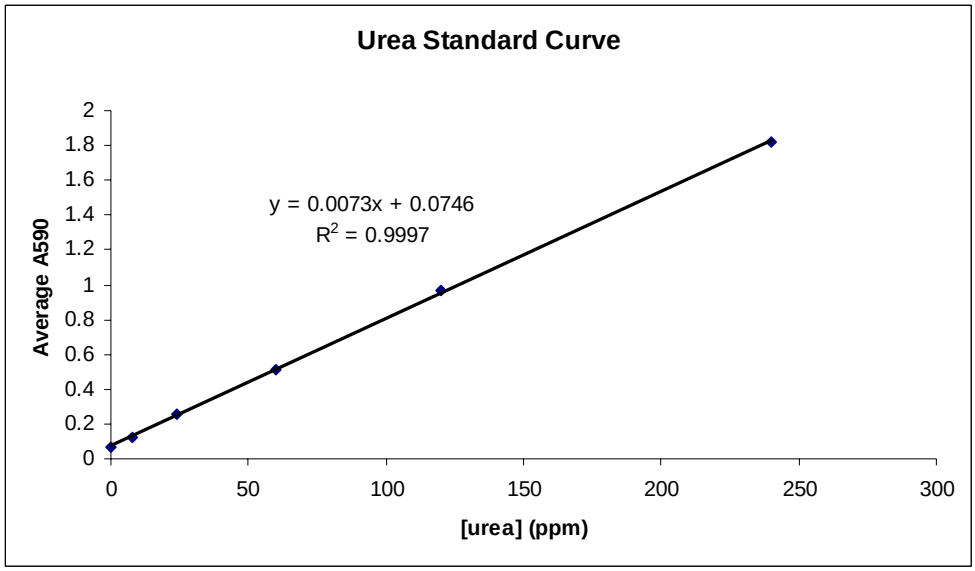
1. Add 5 µL of each standard in duplicate into 30 µL urease solution in microplate wells. Tap plate gently 3-4 times to mix sample and enzyme. Incubate 20 min at room temperature.
2. Add 30 µL of Phenol Prusside and 180 µL 1X Alkaline Hypochlorite to each well. Incubate for 20 min at room temperature.
3. Measure the absorbance of each sample in duplicate at 590 nm.

A plot of average absorbance at 590 nm as a function of urea concentration should provide a tight linear curve. Each of the standard points should be resolved from the other neighboring points.

DATA ANALYSIS

Calculation of Urea Concentration

A standard curve can be constructed by plotting the average absorbance obtained from each reference standard against its concentration in ppm.



Calculate the slope and the y-intercept for the line which fits the standard curve data.

The urea-specific signal (usually about 70% of the total signal) can be determined by subtracting the average absorbance for each milk sample *without* urease from the corresponding average absorbance for each milk sample containing urease.

Average urea specific absorbance
= (average sample absorbance with urease - average sample absorbance without urease)

The urea concentration in the well can be determined using the equation:
Urea concentration = (Average urea specific absorbance-Y intercept) / Slope
Urea concentration in starting sample = Concentration x dilution factor (e.g. 5 for dairy samples; 1 for serum samples).

A special program with Excel functionality, *MaxSignal® Enzymatic Assay Analysis System*, is available upon request to evaluate the test results. Please contact your local distributor or foodfeedsafety@biooscientific.com for further information.

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