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Product Information

Urea Assay Kit

Catalog Number **MAK006**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Urea is the major end product of nitrogen metabolism in most animals and is produced in a series of reactions in the liver called the urea cycle. In the urea cycle, ammonia is converted to urea, which is carried by blood to the kidneys for elimination from the body. High levels of urea in the blood may indicate renal failure. Urea levels may also be elevated in response to treatment with certain drugs such as corticosteroids or in response to decreased kidney filtration due to dehydration or congestive heart failure. Decreased blood urea levels can occur in response to liver disease or malnutrition.

In this assay, Urea concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm) product, proportional to the Urea present.

Components
The kit is sufficient for 100 assays in 96 well plates.

Urea Assay Buffer Catalog Number MAK006A	25 mL
Peroxidase Substrate, in DMSO Catalog Number MAK006B	0.2 mL
Enzyme Mix Catalog Number MAK006C	1 vl
Developer Catalog Number MAK006D	1 vl
Converting Enzyme Catalog Number MAK006E	1 vl
Urea Standard, 100 mM Catalog Number MAK006F	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Keep vials tightly capped when not in use to minimize the uptake of NH_3 from the air.

Urea Assay Buffer – Allow buffer to come to room temperature before use.

Peroxidase Substrate – Thaw at room temperature to melt solution prior to use. Aliquot and store protected from light and moisture at –20 °C.

Enzyme Mix, Developer, and Converting Enzyme – Reconstitute each with 220 µL of Urea Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

<u>Urea Standards for Colorimetric Detection</u>
Dilute 5 µL of the 100 mM (100 nmole/µL) Urea Standard Solution with 995 µL of Urea Assay Buffer to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.5 mM Urea standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add Urea Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Tissue (20 mg) or cells (2×10^6) should be rapidly homogenized in 100 µL of cold Urea Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

Serum and other liquid samples can be directly added to the wells.

Bring samples to a final volume of 50 µL with Urea Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Ammonium ion, NAD^+/$NADP^+$, and pyruvate present in the sample can generate background. To control for background, include a blank sample for each sample by omitting the Converting Enzyme in the Reaction Mix.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mix

Reagent	Sample Blank	Samples and Standards
Urea Assay Buffer	44 µL	42 µL
Peroxidase Substrate	2 µL	2 µL
Enzyme Mix	2 µL	2 µL
Developer	2 µL	2 µL
Converting Enzyme	–	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 60 minutes at 37 °C. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Urea Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Urea standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected measurement. Using the corrected measurement, the amount of Urea present in the sample may be determined from the standard curve.

Concentration of Urea

$$S_a/S_v = C$$

S_a = Amount of Urea in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells
C = Concentration of Urea in sample

Urea molecular weight: 60.07 g/mole

Sample Calculation

Amount of Urea (S_a) = 4.84 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of Urea in sample

$$4.84 \text{ nmole}/50 \text{ µL} = 0.0968 \text{ nmole/µL}$$

$$0.0968 \text{ nmole/µL} \times 60.07 \text{ ng/nmole} = 5.81 \text{ ng/µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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