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ab234052
Urea Assay Kit II

For the measurement of urea level in cells, tissues or biological samples such as urine, serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Urea Assay Kit II (ab234052) is a fast, sensitive and easy to use kit that measures urea level in various tissues/cells. It is based on Jung's method with a modification that delivers more robust and sensitive data. The urea is condensed with o-phthalaldehyde (OPA), followed by a reaction to form a colored product with strong absorbance at 505 nm. The sensitivity of the kit is 10 µM.

Homogenize sample with dH_2O on ice. Centrifuge sample and collect supernatant.

Add sample and standards into a 96-well plate and adjust the volume with dH_2O.

Add reaction mix and incubate for 60 minutes at room temperature.

Measure absorbance at 505 nm.

2. Materials Supplied and Storage

All components in this kit are shipped on blue ice and are suitable for storage at 4°C, unless reconstituted. Upon receipt, immediately store kit at 4°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Probe V	12 mL	4°C	4°C
Reagent I	12 mL	4°C	4°C
Urea Standard	100 µL	4°C or -20°C	4°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer.
- dH_2O water.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Probe V

1. Warm to room temperature before use.

5.2 Reagent I

1. Warm to room temperature before use.

5.3 Urea Standard

1. Dilute Urea Standard to 10 mM (10 nmol/µL) by adding 10 µL of Urea Standard/100 mM Urea Standard to 90 µL dH_2O. Mix well.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Add 0, 2, 4, 6, 8 and 10 µL of the 10 mM Urea Standard into a series of wells in a 96-well plate to generate 0, 20, 40, 60, 80 and 100 nmol/well of Urea Standard.
2. Adjust the volume to 50 µL/well with dH_2O.

7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

Δ Note: For all sample types: To correct for sample interference, spike 2 µL of the 10 mM Urea Standard into each sample well.

7.1 Tissue or cells:

1. Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µL dH_2O on ice.
2. Centrifuge at 10,000 x *g* for 5 minutes and collect the supernatant.
3. Add 1-48 µL supernatant into a 96-well plate and adjust the volume to 50 µL with dH_2O.

7.2 Serum or plasma:

1. Serum and plasma samples can be measured directly.
2. Add 2-10 µL serum or plasma sample into a 96-well plate and adjust the final volume to 50 µL with dH_2O.

7.3 Urine:

1. Centrifuge samples at 10,000 x *g* for 5 minutes at room temperature and collect the supernatant.
2. Dilute the supernatant 50 times by adding 10 µL of supernatant into 490 µL dH_2O.
3. Add 2-10 µL diluted urine sample into a 96-well plate and adjust the volume to 50 µL with dH_2O.

7.4 Other:

1. For other liquid samples that are not clear such as milk, use 10 kDa spin column to clarify and use filtrate to measure the urea content.

Δ Note: For unknown samples, we suggest testing several doses to ensure the reading are within the Standard Curve range.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

1. Mix enough reagents for the number of assays (samples and standards) to be performed.
2. For each well, prepare 200 µL of Reaction Mix containing:

Component	Reaction Mix (µL)
Probe V	100 µL
Reagent I	100 µL

3. Add 200 µL of the Reaction Mix into each standard and sample well. Mix well.
4. Incubate for 60 minutes at room temperature.
5. Measure absorbance (OD 505 nm).

9. Data Analysis

1. Subtract the mean value of the blank (Standard 0) from all readings. This is the corrected absorbance.
2. Plot the Urea Standard Curve.
3. Correct for any interference by subtracting the sample reading from spiked sample reading.
4. Calculate the Urea amount (X) in the sample wells:

$$X\ (nmol) = \left(\frac{(ODsample\ corrected)}{\big(ODsample + 20(corrected)\big) - (ODsample\ corrected)}\right) * 20\ nmol$$

Sample Urea concentration (C) = X/V x D = nmol/µl = µmol/ml or mM

Where:

X = amount of Urea (nmol) in the sample well

V = sample volume added in reaction well (µL)

D = Sample Dilution Factor

To convert sample urea concentration to mg/dL, multiply C by 6.006.

Urea MW: 60.06 g/mol

To express as BUN (blood urea nitrogen):

BUN = C * 2.8011 (mg/dL)

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only**.

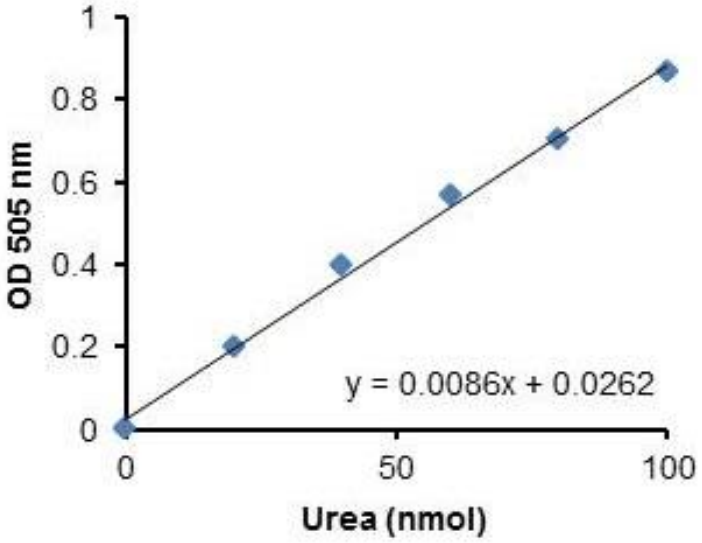

Figure 1. Urea Standard Curve.

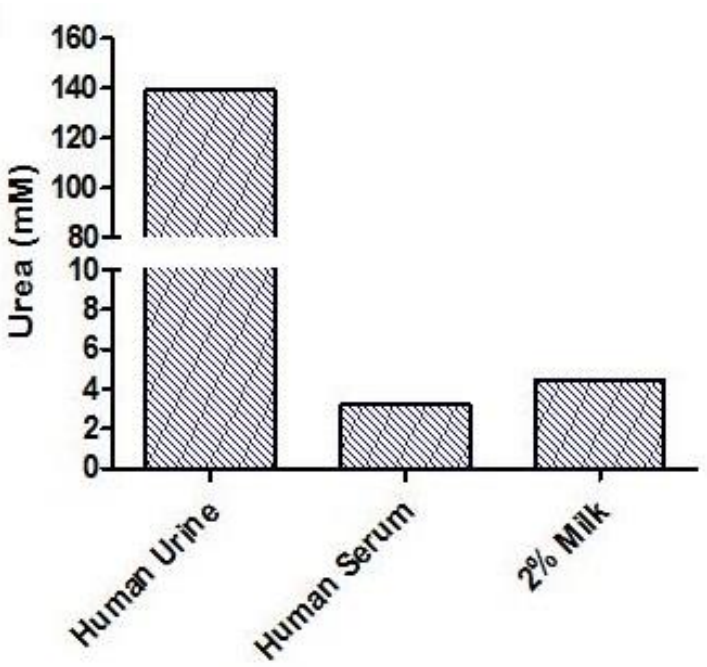

Figure 2. Measurement of urea in human urine (5 µL of 50 times diluted), human serum (5 µL) and 2% milk (20 µL). Assays were performed following the kit protocol.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)