ab204697

Urease Activity Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate measuring of Urease activity in biological samples and soil.

View kit datasheet: www.abcam.com/ab204697
(use www.abcam.cn/ab204697 for China, or www.abcam.co.jp/ab204697 for Japan)

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

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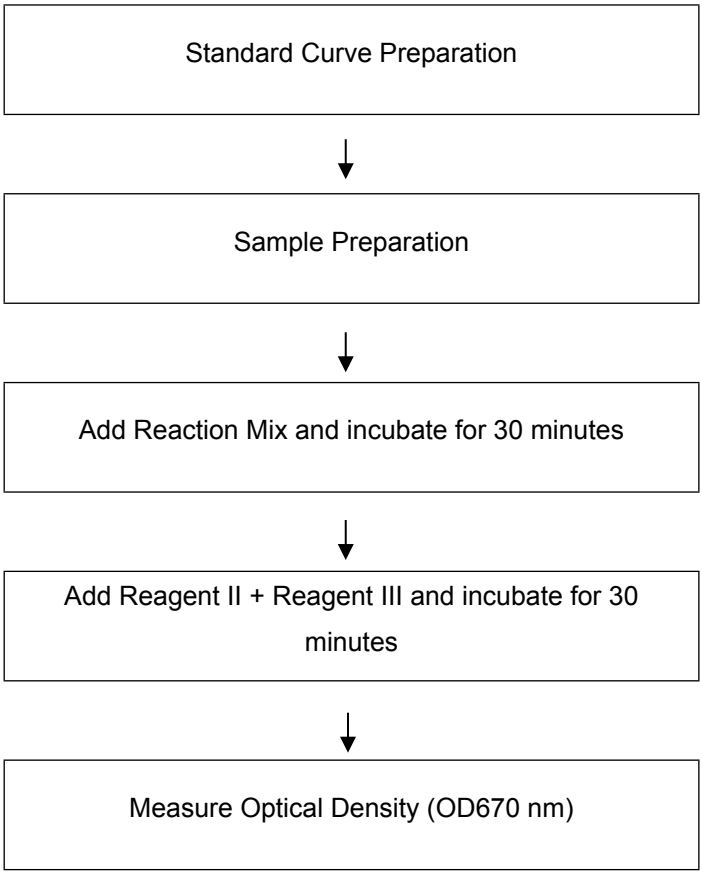
1. BACKGROUND

Urease Activity Assay Kit (Colorimetric) (ab204697) provides a quick and easy way to determine urease activity. The kit uses a modified Berthelot method to detect ammonia, which can be measured at 670 nm. The kit is suitable for measuring urease activity of biological and soil samples as well as of purified enzyme. The limit of detection is below 0.001 mU.

Urease (EC 3.5.1.5) catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Urease is found in certain bacteria, yeast, fungi, and plants. In addition, it is found environmentally in soil samples and fecal matter. Due to ammonia production, urease can play a role in the pathogenesis of certain medical conditions including urinary stones, peptic ulcers, and hepatic encephalopathy. As such, urease can be used as a diagnostic for gastrointestinal and urinary tract pathogens.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Urease Assay Buffer	15 mL	-20°C	-20°C
Reagent II	8 mL	-20°C	-20°C
Reagent III	4 mL	-20°C	-20°C
50X Urea Solution	1.5 mL	-20°C	-20°C
Converter Mix D	1 Vial	-20°C	-20°C
Ammonium Standard II	100 µL	-20°C	-20°C

PLEASE NOTE: Ammonium Standard II was previously labelled as Ammonium Chloride Standard (100 mM), and Reagent II as Ammonia Reagent 1, and Reagent III as Ammonia Reagent 2, and Converter Mix D as Urease, and Converter Enzyme V, and 50X Urea Solution as Urea (50x). The composition has not changed.

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter OD = 670 nm
- 96 well plate: clear plate with flat bottom
- Heat block or water bath
- 50 mM sodium acetate, pH 5 (for soil sample preparation only)
- protease inhibitors or Protease Inhibitor Cocktail (ab65621)

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Urease Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 **Reagent II:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **Reagent III:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 **Ammonium Standard II/Ammonium Chloride Standard (100 mM):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.5 **50X Urea Solution:**

Make 1X Urea solution by diluting 1:50 in ddH_2O. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Equilibrate to room temperature before use.

9.6 **Converter Mix D:**

Dissolve in 220 µL Urease Assay Buffer. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw. Stable for two months. Keep on ice during use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare a 1 mM Ammonium Standard II/Ammonium Chloride standard by diluting 5 µL of the 100 mM Ammonium Standard II/Ammonium Chloride standard with 495 µL of ddH_2O.

10.2 Using 1 mM Ammonium Standard II/Ammonium Chloride standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	End conc. Am Chl in well (nmol/100µL/well)
1	0	300	100	0
2	12	288	100	4
3	24	276	100	8
4	36	264	100	12
5	48	252	100	16
6	60	240	100	20

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- To relate urease activity to protein amount, measure protein concentration using a BCA protein quantification assay.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold PBS with protease inhibitors.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample for 10 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.2 Recombinant or purified enzyme:

11.2.1 Dissolve in Urease Assay Buffer.

11.3 Soil samples:

11.3.1 Homogenize 0.5 g soil sample in 10 mL of 50 mM sodium acetate, pH 5 for 2 minutes.

11.3.2 Stir vigorously and add 0.9 mL into a clean tube as test sample and 0.2 mL into another tube as soil background control.

11.3.3 Centrifuge soil background control tube at 8000 x g for 1 minute.

11.3.4 Collect 0.1 mL supernatant and add supernatant into desired well in a 96-well plate. Proceed directly to step 12.5 (develop and measure) immediately.

11.3.5 To test sample, add 0.1 mL of 1x Urea Solution and incubate at 37°C for 2 hrs. Centrifuge at 8000 x g for 1 minutes. Collect 0.1 mL supernatant and add into desired well in a 96-well plate. Proceed directly to step 12.5.

NOTE: *For soil samples, we suggest testing several incubation lengths (1 - 24 hours) to ensure activity is within the assay range.*

11.4 **Positive Control:**

11.4.1 Dilute Converter Mix D 1:10 by adding 10 µL Converter Mix D into 90 µL Urease Assay Buffer.

NOTE: *For samples with unknown urease activity, we suggest testing several amounts of enzyme or cell homogenate to ensure the activity is within the assay range. Alternatively, for limited or fixed sample amounts, incubation time in Step 12.4 can be increased.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 100 µL standard dilutions.
- Sample wells = 1 – 10 µL samples (adjust volume to 10 µL/well with ddH_2O).
- Background control wells= 10 µL/well Urease Assay Buffer.
- Positive control = 2 - 10 µL of diluted Converter Mix D (from Step 11.4) (adjust volume to 10 µL/well with ddH_2O).

12.2 **Reaction Mix:**

Prepare 90 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
Urease Assay Buffer	**88**
Urea Solution/Urea (1X)	**2**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 90 µL of Reaction Mix into sample and control wells.

DO NOT add reaction mix to Standards or soil sample.

12.4 Mix and incubate at 37°C for 30 minutes (or longer if there is limited amount of enzyme in the sample).

12.5 Add 80 µL of Reagent II into each well and mix.

12.6 Add 40 µL of Reagent III into each well and mix.

12.7 Incubate at 37°C for 30 minutes.

12.8 Measure output on a colorimetric microplate reader at OD670.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from positive control and sample reading.

For soil samples, subtract soil background control reading from soil sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final amount of ammonia generated by urease during the reaction time.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Urease activity (nmol/mg protein/min or mU/mg protein) in the test samples is calculated as:

$$Urease\ activity = \left(\frac{B}{T * P}\right)$$

Where:

B = Amount of ammonia from Standard Curve (nmol)

P = Protein concentration (mg).

T = Reaction time (min).

13.7 For soil samples, the Urease activity (nmol/mg soil/hour) is calculated as:

$$Soil\ Urease\ activity = \left(\frac{B}{T * 5}\right)$$

Where:

B = Amount of ammonia generated in the sample well from Standard Curve (nmol)

5 = mg of soil measured in the assay

T = Reaction time (hour)

Unit Definition:

1 Unit Urease activity = amount of Urease which generates 1.0 µmol of ammonia per minute at pH 7 at 37°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

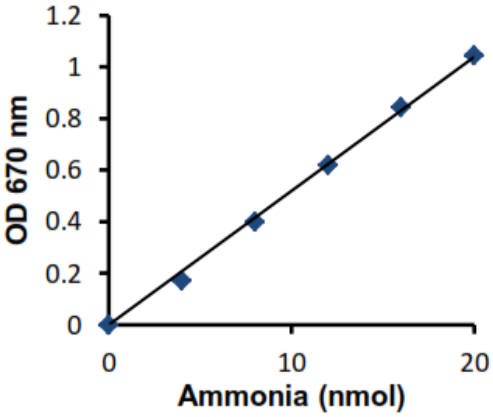


Figure 1. Typical ammonia Standard calibration curve

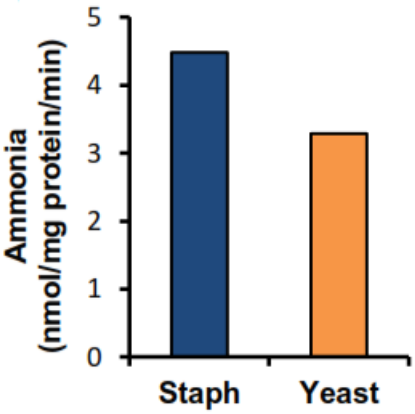


Figure 2. Measurement of Urease activity in *Staphylococcus aureus* and *Saccharomyces cerevisiae* (yeast) cell extracts.

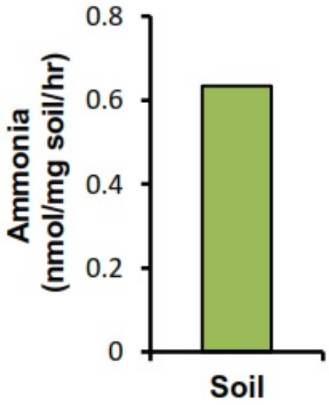


Figure 3. Measurement of Urease activity in soil.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, positive control and prepare reaction mix (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (100 µL), samples (10 µL), positive control (10 µL) and background wells (10 µL).
- Prepare Urease Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)
Urease Assay Buffer	**88**
Urea Solution/Urea	**2**

- Add 90 µL of Urease Reaction Mix to the standard and sample wells.
- Incubate plate at 37°C 30 minutes.
- Add 80 µL of Reagent II into each well.
- Add 40 µL of Reagent III into each well.
- Incubate plate at 37°C 30 minutes.
- Measure plate at OD 670 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

18. 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)