ab83362 - Urea Assay kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Urea levels in various samples.
This product is for research use only and is not intended for diagnostic use

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt. 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. Reconstituted components are stable for 2 months

Materials Supplied:

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 14	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Developer Mix A	1 vial	-20°C	-20°C
Developer Mix L	1 vial	-20°C	-20°C
Converter Mix D	1 vial	-20°C	-20°C
Urea Standard	100 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 14 was previously labelled as Assay Buffer XIV and Urea Assay Buffer, Developer Mix A as Development Enzyme Mix I and Enzyme Mix, Developer Mix L as Developer I and Developer, and Converter Mix D as Converter Enzyme V and Converting Enzyme. Only the component names have changed, the mechanism of detection has not changed.

Materials Required, Not Supplied

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

- MilliQ water or other type of double distilled water (ddH2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD570 nm
- 96 well plate: clear plates for colorimetric assay
- Orbital shaker
- Dounce homogenizer or pestle (if using tissue)

Limitations

- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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.

- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at RT before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes/plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

Reagent Preparation:

Briefly centrifuge small vials at low speed prior to opening. **All solutions should be kept capped when not being used to prevent absorption of NH_3 from the air.**

Assay Buffer 14: Ready to use as supplied. Equilibrate to RT before use. Store at -20°C and protect from light. If precipitant forms, sonicate to bring back into solution.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

NOTE: DMSO tends to be solid when stored at -20°C, even when left at RT, so it needs to melt for few minutes at 37°C. Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C protected from light and moisture. Keep on ice while in use.

Developer Mix A, Developer Mix L, Converter Mix D: Reconstitute separately in 220 µL Assay Buffer 14. Aliquot Developer Mix A so that you have enough volume to perform the desired number of tests. Store at -20°C. Use within two months. **Keep on ice while in use**.

Urea Standard: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Aliquot standard so that you have enough volume to perform the desired number of tests. Store at -20°C.

Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

1. Prepare a 0.5 mM Urea standard by diluting 5 µL of 100mM Urea Standard in 995 µL of ddH2O.
2. Using 0.5 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL)

Standard #	Volume of Standard (µL)	Assay Buffer 14(µL)	Final volume standard in well (µL)	End [Urea] in well
1	0	125	50	0 nmol/well
2	5	120	50	1 nmol/well
3	10	115	50	2 nmol/well
4	15	110	50	3 nmol/well
5	20	105	50	4 nmol/well
6	25	100	50	5 nmol/well

Sample Preparation

We recommend performing several dilutions and different volumes of sample to ensure readings are within the standard value range and that you use fresh samples. If you cannot perform the assay at the same time, we complete the Sample Preparation step before storing samples. If that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples at -80°C. When you are ready to test your samples, thaw them on ice. This might affect the stability of your samples - readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (recommendation = 2 x 10^6 cells).
2. Wash cells with cold PBS and resuspend cells in 100 µL Assay Buffer 14.
3. Homogenize cells using, for example, a dounce, a sonicator or passing through a needle.
4. Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a clean tube and keep on ice.
6. As guidance, we recommend using a volume that equals 5 - 20 x 10^4 cells/well. Bring volume to 50 µL/well with Assay Buffer 14.

Tissue:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 20 mg tissue and wash tissue in cold PBS.
2. Resuspend tissue in 100 µL of Assay Buffer 14.
3. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 - 15 passes.
4. Centrifuge samples for 2- 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a clean tube.
6. As guidance, we recommend using 1 – 25 µL of tissue extraction, which corresponds approximately to 10 – 50 µg/well of protein. Bring volume to 50 µL/well with Assay Buffer 14.

Serum, plasma and urine samples:

1. Samples can be tested directly by adding sample to the wells.
2. We recommend using 1-25 µL of plasma, serum, urine or other liquids.

3. Cell culture medium:

1. Harvest culture medium from cells grown to confluency
2. Asses in a range of dilutions (1/2 – 1/1000)

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.

Set up Reaction wells:

1. Standard wells = 50 µL Standard dilutions.
2. Sample wells = 1 – 25 µL samples (adjust volume to 50 µL/well with Assay Buffer 14.
3. Background control sample wells = 0.1 – 25 µL samples (adjust volume to 50 µL/well with Assay Buffer 14. NOTE: for samples containing ammonium ion, NAD+/NADP+ or pyruvate, as they can generate background.

Reaction Mix:

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 14	42	44
OxiRed Probe	2	2
Developer Mix A	2	2
Developer Mix L	2	2
Converter Mix D	2	0

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation: **X µL component x (Number samples + standards +1).**

1. Add 50 µL of Reaction Mix into each standard and sample wells.
2. Add 50 µL of Background Reaction Mix into background sample control wells.
3. Mix and incubate at room temperature for 30 minutes protected from light.

NOTE: This kit is prone to increase background at higher incubation temperatures.

4. Measure output on a microplate reader (OD570 nm). Alternatively, the assay may be read in kinetic mode.
   ***NOTE: Once the reaction is complete, continued incubation will increase background.**

Calculations

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates). If readings were taken in kinetic mode it is best to use a time point directly after the samples and standard curve has plateaued. Background will continue to increase once the reaction is completed.

1. Average the duplicate reading for each standard and sample. If the sample background control is significant, subtract the sample background from sample reading.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of Urea.
4. Use linear regression to plot the line of best fit. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation.

5. Interpolate sample amounts of rea from the standard curve plotted using the following equation: $\text{Sa} = \left(\frac{\text{Corrected absorbance} - (\text{y intercept})}{\text{Slope}}\right)$
6. Concentration of urea in test samples is calculated as:

$$\text{Urea Concentration} = \left(\frac{\text{Sa}}{\text{Sv}}\right) * \text{D}$$

Where:

- Sa = Sample amount (nmol) from standard curve.
- Sv = Sample volume (µL) added into the wells.
- D = Dilution factor of sample

Quick assay procedure

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing this assay for the first time**. All solutions should be kept capped when not being used to prevent absorption of NH3 from the air.**

NOTE: Failing to keep enzymes on ice will result in higher backgrounds. Do not make reaction mix or background reaction mix until ready to use. Solubilize Developer Mix A, thaw OxiRed probe and all other components (aliquot if necessary); get equipment ready.

- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Urea Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 14	42	44
OxiRed Probe	2	2
Developer Mix A	2	2
Developer Mix L	2	2
Converter Mix D	**2**	**0**

- Add 50 µL of Urea Reaction Mix into standard and sample wells and mix.
- Add 50 µL of Background Reaction Mix to background wells and mix.
- Incubate plate at room temperature for 30 minutes.
- Measure plate at OD570 nm.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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