ab83360 – Ammonia Assay Kit

For the measurement of total ammonia and ammonium levels in various samples
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab83360
(use www.abcam.cn/ab83360 for China, or www.abcam.co.jp/ab83360 for Japan)

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability: Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze-thaws of reagents.

Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XIV/Ammonia Assay Buffer	25 mL	-20°C	-20° C
OxiRed Probe	200 µL	-20°C	-20°C
Developer III/Developer	1 vial	-20°C	-20°C
Development Enzyme Mix I/Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Converter Mix I/Converting Enzyme (Lyophilized)	1 vial	-20°C	-20°C
Ammonium Standard (10 mM)/NH_4Cl Standard (10 mM)	100 µL	-20°C	-20°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 570 nm
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)

Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.
ΔNote: All solutions in this kit must be kept capped when not in use to prevent absorption of ammonia from the air especially the OxiRed Probe. Setting up the assay in a laminar flow hood would also reduce the chances of contamination with Ammonia.

Assay Buffer XIV/Ammonia Assay Buffer: Ready to use as supplied. Equilibrate to Room temperature (RT) before use.

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. Protect from light, air and moisture as the probe is extremely sensitive.
ΔNote: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C.

Developer III/Developer: Reconstitute in 220 µL Assay Buffer XIV/Ammonia Assay Buffer. Aliquot Developer III/developer so that you have enough volume to perform the desired number of assays and prevent multiple freeze-thaws. Store at -20°C. Use within two months.

Development Enzyme Mix I/Enzyme Mix: Reconstitute in 220 µL Assay Buffer XIV/Ammonia Assay Buffer. Aliquot the mix so that you have enough volume to perform the desired number of assays and prevent multiple freeze-thaws. Store at -20°C. Use within two months.

Converter Mix I/Converting Enzyme: Reconstitute in 220 µL Assay Buffer XIV/Ammonia Assay Buffer. Aliquot Converter Mix I/converting enzyme so that you have enough volume to perform the desired number of assays and prevent multiple freeze-thaws. Store at -20°C. Use within two months.

Ammonium Standard (10 mM)/Ammonium chloride (NH4Cl) Standard: Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

Standard Preparation

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

- Prepare 100 µL of 1 mM Ammonium Standard (10 mM)/Ammonium Chloride (NH4Cl) Standard by adding 10 µL of the 10 mM Ammonium Chloride Standard to 90 µL of ddH2O.
- Using 1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

Standard #	Ammonium Standard (1 mM)/NH_4Cl Standard (µL)	Assay Buffer XIV/Assay Buffer (µL)	Final volume standard in well (µL)	End amount of NH_4Cl in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Sample Preparation

- 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 for the most reproducible assay. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them 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. Avoid multiple freeze-thaws.

Cells (adherent or suspension) samples:

1. Harvest the number of cells necessary for each assay (initial recommendation = 2 x 106 cells – equivalent of 1-5 x 104 cells/well required).
2. Wash cells in cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer XIV/Assay Buffer.
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge sample for 2-5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

ΔNote: Phenol red may interfere with the assay if the medium contains enough to add red color to your sample. Typically, when diluted medium is used and the well is made up to 50 µL with buffer, it will not interfere.

Tissue Samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg (equivalent to 20-50 µg/well).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 100 µL of Assay Buffer XIV/Assay Buffer.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
5. Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Plasma Samples:

1. Collect whole blood into heparin tubes. Keep sample at 4°C during preparation.
2. Remove cells by centrifuging sample for 10 minutes at 1,000 x g at 4°C.
3. Collect supernatant and transfer to a clean tube. After centrifugation, it is important to immediately transfer into a clean tube.
4. Keep on ice.

Initial sample recommendation = 5 – 15 µL/well of plasma

Urine and other biological fluids:

1. Test directly in the assay.

Initial sample recommendation = <0.5 µL of urine (100x dilution in Assay Buffer XIV/Assay Buffer).

ΔNote: Samples can be stored frozen at -20°C to - 80°C after lysis/ homogenization with Assay Buffer XIV/assay buffer.

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.

Δ Note: This product is very sensitive, and reagents can react with other sources of ammonia present in the laboratory. Ensure you keep the plate closed with a lid when not pipetting, and work in a glovebox or negative air pressure area if possible.

Δ Note: If the reaction mix turns pink upon preparation this indicates ammonia contamination from the environment as the probe can become oxidized leaving it slightly pink. High background readings with the blank typically indicates absorption of ammonia into the reagents, try using les probe than recommended.

Δ Note: Pyruvate in samples will interfere with the assay. If a significant amount of pyruvate is suspected in your samples, set up Sample Background Controls. To correct for background, pyruvate readings must be subtracted from sample readings.

Δ Note: The ammonia/ammonium content analyzed with this kit is pH dependent. At physiological pH, nearly all ammonia will exist as ammonium in solution. The chemical equation that drives the relationship between ammonia and ammonium is:

$$NH3 + H2O \leftrightarrow NH4+ + OH-$$

When the pH is low, the reaction is driven to the right, and when the pH is high, the reaction is driven to the left. In general, at around room temperature, at a pH less than 6.0, the proportion of ammonium-N plus ammonia-N as NH3 is very-very low and as NH4+ is very-very high. At a pH around 8.0 (the Assay Buffer XIV/assay buffer pH), the proportion as NH3 is 10 percent or less. Laboratory methods measure ammonium-N plus ammonia-N. It is very difficult to directly determine the activity of aqueous ammonia, so instead the surrogate of ammonium-N plus ammonia-N is used.

Plate Loading:

For colorimetric use clear plates and for Fluorometric use black walled, clear bottom plates.

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 - 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XIV/Ammonia Assay Buffer).
- Sample Background Control wells = 2 - 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XIV/Ammonia Assay Buffer).

Ammonia reaction mix:

1. Prepare 50 µL of Ammonia Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency.
2. Add 50 µL of Reaction Mix into each standard and sample well.
3. Add 50 µL of Background Reaction Mix into the background control sample wells.
4. Mix and incubate at 37°C for 60 minutes protected from light.
5. Measure output immediately on a colorimetric microplate reader at OD 570 nm.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XIV/Ammonia Assay Buffer	42	44
OxiRed Probe	2	2
Development Enzyme Mix I/Enzyme Mix	2	2
Developer III/Developer	2	2
Converter Mix I/Converting Enzyme	2	0

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected absorbance.
3. Subtract the sample background control from sample readings.

4. Plot the corrected values for each standard as a function of the final concentration of ammonia.
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).
6. Apply the corrected sample OD reading to the standard curve to get ammonia (B) amount (nmol) in the sample wells.
7. Concentration of ammonia and ammonium in nmol/µL (mM) in the test samples is calculated as:

$$Ammonia\ and\ ammonium\ concentration = \frac{B}{V} * D$$

Where:

B = amount of ammonia and ammonium in the sample well calculated from standard curve in nmol.

V = sample volume added in the sample wells (µL).
D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

NH4+ Molecular Weight = 18.04 g/mol.

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)