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:
http://www.abcam.com/ab83360 (use http://www.abcam.cn/ab83360 for China, or http://www.abcam.co.jp/ab83360 for Japan)

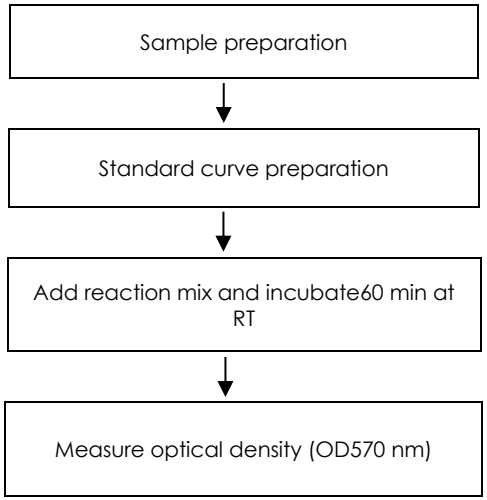
Background:

Ammonia Assay Kit (ab83360) provides a simple, rapid and sensitive method for accurate quantification of ammonia in a variety of biological samples such as tissue extracts, cell lysates, cell culture media, urine, plasma, serum, and other biological fluids.

Ammonia or ammonium is converted to a product that reacts with the OxiRed probe to generate color (OD 570 nm) which can be easily quantified using a plate reader. The kit can detect as little as 1 nmol (~20 µM) of ammonia or ammonium and is much more sensitive than quantification using an NADPH based method.

Assay Summary:

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



QUICK ASSAY PROCEDURE

- Set plate reader to Room Temperature
- Solubilize Developer Mix U, Developer Mix A and Converter Mix A, thaw OxiRed™ Probe, Ammonia Standard and Ammonia Assay Buffer (aliquot if necessary)
- Prepare standard curve
- Prepare samples in duplicate
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Ammonia Reaction Mix (Number samples + standards + 1).
- Add 50 µL Reaction Mix to each well.
- Incubate plate for 60 minutes at room temperature
- Measure plate at OD 570nm

Precautions & Limitations:

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.
- 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.

Extreme care should be taken to ensure that no ammonia vapors (e.g. many lab and household cleaners contain ammonia) are in the laboratory air where this assay is to be performed. Ammonia vapors in the air will be rapidly absorbed by kit components resulting in very high background making the kit unusable. Laboratories where ammonia and ammonia based cleaners are used routinely in cleaning and disinfecting are not appropriate places to run this assay.

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. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 14	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Developer Mix U	1 vial	-20°C	-20°C
Developer Mix A	1 vial	-20°C	-20°C
Converter Mix A	1 vial	-20°C	-20°C
Ammonium Standard I	0.1 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 14 was previously labelled as Assay Buffer XIV and Ammonia Assay Buffer, Developer Mix U as Developer III and Developer, Developer Mix A as Development Enzyme Mix I and Enzyme Mix, and Converter Mix A as Converter Mix I and Converting Enzyme. Only the component name has changed, the kit 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:

- Microplate reader capable of measuring absorbance at OD 570 nm
- 96 well clear plate with clear flat bottom
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using cells or tissue)
- 1x PBS pH 7.4
- MilliQ water or other type of double distilled water (ddH2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.
- Avoid repeated freeze-thaws of reagents.

Note: All solutions in this kit must be kept capped when not in use to prevent absorption of ammonia from the air. Setting up the assay in a laminar flow hood will reduce the chances of contamination with ammonia.

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

Ammonium Standard: is ready to use as supplied. Keep Ammonium Standard on ice while in use. Store at -20°C and protect from light.

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. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

Developer Mix U, Developer Mix A and Converter Mix A: Reconstitute each with 220 µL Assay Buffer 14. Keep on ice while in use. Aliquot and use within two months.

Sample Preparation:

1. We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
2. 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 at -80°C. Alternatively, 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.
3. Interferences: 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.

Cells (adherent or suspension) samples:
1. Harvest the number of cells necessary for each assay (initial recommendation = 2 x 10^6 cells - equivalent of 1-5 x 10^4 cells/well required).
2. Wash cells in cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer 14.
4. Homogenize cells with a Dounce homogenizer sitting on ice
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.

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 14.
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.

Liquid Samples (Urine and other biological fluids): Samples can be diluted in Assay Buffer for testing. Initial sample recommendation = < 0.5 µL of urine (100X dilution in Assay Buffer 14.)

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 standard to set up duplicate readings (2 x 50 µL).

Prepare working dilution of Ammonium Standard I as follows:

1. 1 mM dilution: Add 10 µL of 10 mM Ammonium Standard I to 90 µL ddH_2O. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
2. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer 14 to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Chloride Standard or, prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves):

Standard #	Volume of 1 mM Ammonium Standard (µL)	Assay Buffer 14 (µL)	Final volume standard in well (µL)	End NH_4Cl Amount (nmol/well)
1	0	125	50	0
2	5	120	50	2
3	10	115	50	4
4	15	110	50	6
5	20	105	50	8
6	25	100	50	10

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.
- We recommend testing several dilutions or doses of your sample to ensure the readings are within the standard value range.

1. Set up Reaction wells:
   - Standard wells = 50 µL standard dilutions.
   - Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 14.
   - Background control wells = 2 – 50 µL samples (adjust volume to 50 µL Assay Buffer 14.
2. Each well (standards, samples, and controls) requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +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 U	2	2
Converter Mix A	2	0

3. Mix Master Reaction Mixes by inversion. Add 50 µL of the Master Reaction Mix to each standard and sample well. Add 50 µL of the Master Background Reaction Mix to each sample background well. Use a clean tip for each well.
4. Mix and incubate at room temperature for 60 minutes, protected from light.
5. Measure absorbance immediately on a microplate reader at OD 570 nm. Alternatively, the assay may be read in kinetic mode.

***NOTE: Once the reaction is complete, continued incubation will increase background.**

Calculations:

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard values. This is the corrected absorbance.
3. Subtract blank (standard #1) from sample readings. If the sample background control is higher than the blank, subtract this from the sample reading instead of the blank. This is the corrected sample absorbance.
4. Plot the corrected absorbance values for each standard as a function of the final amount of ammonia in nmoles.
5. Calculate the equation of the standard curve using a linear regression and determine the slope.
6. Apply the corrected sample absorbance to the standard curve to get ammonia (B)amount (nmoles) in the sample wells using the following equation:

$$B = \left(\frac{Corrected\ sample\ absorbance - (y - intercept)}{Slope}\right)$$

7. Concentration of Ammonia in the test samples is calculated as:

$$Ammonia\ /\ Ammonium\ Concentration = \frac{B}{V} \times D = nmol/\ \mu L = mM$$

Where:
B = amount of Ammonia and Ammonium in the sample well calculated from standard curve (in nmoles).
V = amount of sample volume added in sample wells (µL).
D = sample dilution factor (prior to reaction well set up).

NH_4^+ Molecular Weight = 18.04 g/mol.

FAQs:

Q. Why did my reaction mix turn pink before adding it to my samples and standard?

A. 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. Additionally, increased time and temperature will contribute to background innate to the system.

Q. Does this kit measure ammonium also?

A. 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:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

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 NH_3 is very-very low and as NH_4^+ is very-very high. At a pH around 8.0 (the Assay Buffer 14/assay buffer pH), the proportion as NH_3 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.

Q. Why did my reaction mix turn pink?

A. 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 less probe than stated in the protocol. The reaction also has some innate background that increases with time and temperature.

Q. Why do we need sample background controls?

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

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:

www.abcam.com/contactus
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