

ab102509 – Ammonia Assay Kit: Modified Berthelot (Colorimetric)

For rapid, sensitive and accurate measurement of ammonia 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/ab102509> (use <http://www.abcam.cn/ab102509> for China, or <http://www.abcam.co.jp/ab102509> for Japan)

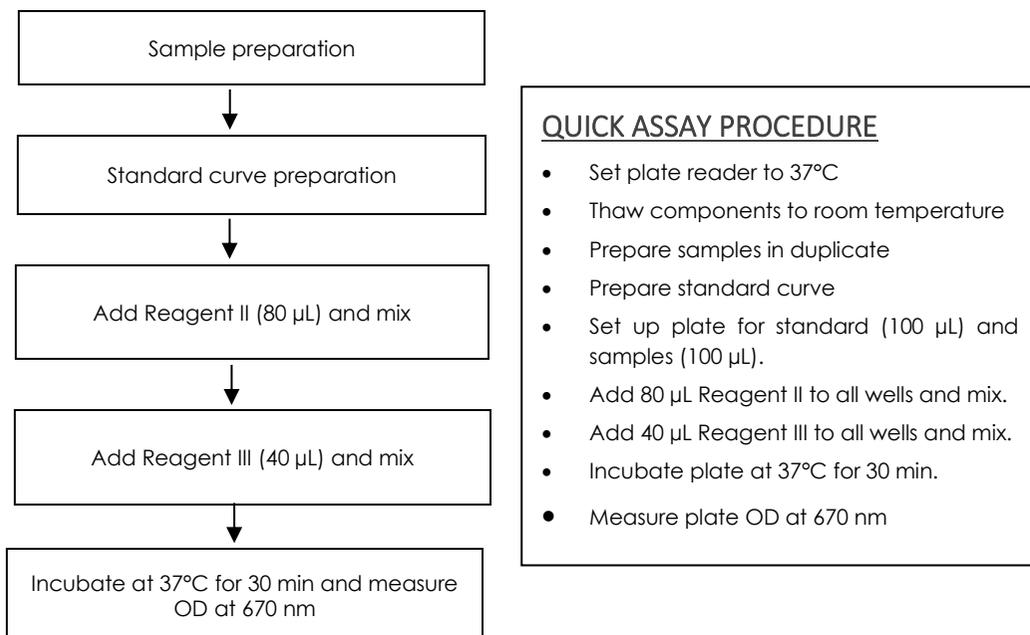
Background:

Ammonia Assay Kit (Colorimetric) (ab102509) provides a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia or Ammonium. Sample types compatible with this assay include cell culture media, urine, plasma, serum, and other biological fluids.

In this non-enzymatic assay, ammonia or ammonium is used to form indophenol, a highly colored product easily quantifiable by colorimetry (OD 670 nm) using a plate reader. The kit can detect less than 1 nmol (~10 μ M) ammonia or ammonium using either a 96 well or 384 well microwell plate.

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.



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 -20°C 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)
Reagent II	8 mL	-20°C
Reagent III	4 mL	-20°C
Ammonium Standard II	100 μ L	-20°C

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 (OD) at 670 nm (colorimetric)
- 96 well clear plate with clear flat bottom (colorimetric assay)
- Orbital shaker
- Microcentrifuge
- MilliQ water or other type of double distilled/deionized water (ddH₂O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Reagent II, Reagent III and Ammonium Standard II are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light and moisture.

Note: All solutions should be kept tightly capped when not in use to prevent absorption of ammonia from the air.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
1. 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, 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.
 2. Interferences:
 - Amines and amides may interfere with the Berthelot assay and should be tested for interference if significant concentrations are expected in samples.
 - The reagents used for the Ammonia assay were selected to minimize interference from non-ammonia sources.

Liquid Samples (Biological): Liquid samples such as cell culture media, plasma, serum, urine, and other biological fluids can be tested directly or after dilution in ammonia-free water. Use a total of 100 µl sample/well in a 96-well plate.

NOTE: biological samples should be deproteinized prior to testing. We recommend using a 10kDa spin column such as ab93349 rather than using acid precipitation to remove proteins as acid can deamidate proteins leading to higher ammonia background levels.

Liquid Samples (Non-biological): Liquid samples such as sea water, soil extracts, etc. can be tested directly or after dilution in ammonia-free water. Use a total of 100 µl sample/well in a 96-well plate.

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 100 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare 1mM dilution of Ammonium Standard II as follows:

1. 1 mM dilution: Add 10 µL of undiluted Ammonium Standard II to 990 µL ddH₂O. 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 100 µl/well with ddH₂O to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Standard II 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 Standard (µL)	ddH ₂ O (µL)	Final volume standard in well (µL)	End Ammonia Amount (nmol/well) Colorimetric Assay
1	0	250	100	0
2	5	245	100	2
3	10	240	100	4
4	15	235	100	6
5	20	230	100	8
6	25	225	100	10

Assay Procedure:

- 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.
1. Set up Reaction wells:
 - Standard wells = 100 µL standard dilutions.
 - Sample wells = 1-100 µL samples (adjust volume to 100 µL/well with ddH₂O).
 2. Add 80 µl of Reagent II to each standard and sample well. Use a clean tip for each well and mix by pipetting.
 3. Add 40 µl of Reagent III/Reagent 2 to each standard and sample well. Use a clean tip for each well and mix by pipetting.
Note: **Do not** make a master mix of Reagent II and Reagent III. Add reagents sequentially.
 4. Incubate at 37°C for 30 minutes, protected from light.
 5. Measure output on a microplate reader at OD 670 nm.

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 and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final amount of ammonia.
4. Calculate the linear equation of the standard curve.
5. Apply the corrected sample absorbance to the standard curve to find (*B*) ammonia/ammonium amount (nmoles) in the the sample wells using the calculation below:

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

6. Concentration of Ammonia/ammonium in the test samples is calculated as:

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

Where:

B = amount of Ammonia in the sample well calculated from standard curve (nmol).

V = volume of sample added in sample wells (μL).

D = sample dilution factor (prior to reaction well set up).

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

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