ab102509

Ammonia Assay Kit – Modified Berthelot – (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Ammonia in various samples.

View kit datasheet: www.abcam.com/ab102509
(use www.abcam.cn/ab102509 for China, or www.abcam.co.jp/ab102509 for Japan)

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

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.

Version 4a, Last Updated 21 December 2023

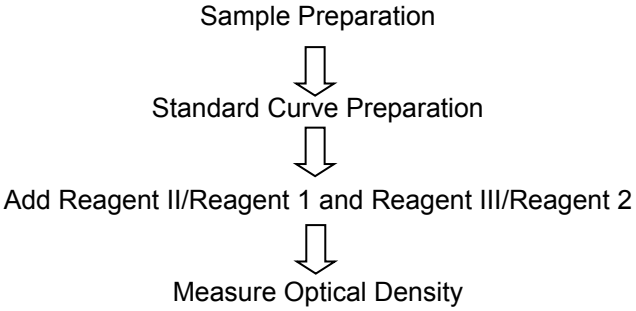
Table of Contents

1. Overview

Ammonia is an important source of nitrogen for living systems. Nitrogen is required for the synthesis of amino acids, which are the building blocks of protein. Ammonia is a metabolic product which is created through amino acid deamination. It plays an important role in both normal and abnormal animal physiology such as acid/base balance.

Abcam provides a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia or Ammonium. In this non-enzymatic assay, ammonia or ammonium is used to form indophenol, a highly colored product easily quantifiable by colorimetry (OD_{670nm}) using a plate reader. The kit can detect less than 1 nmol (~10 µM) ammonia or ammonium using either a 96 well or 384 well microwell plate.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
Reagent II/Ammonia Reagent 1	8 mL
Reagent III/Ammonia Reagent 2	4 mL
Ammonium Standard II/Ammonium Chloride Standard (100 mM)	100 µL

4. Storage and Stability

Upon arrival, store the kit at +4°C.

Read the entire protocol before performing the assay.

The two Ammonia reagents provided are ready to use as supplied.

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

5. Materials Required, Not Supplied

- Distilled water (ddH_2O) or MilliQ
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

6. Assay Protocol

1. Sample Preparation:

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

b) Biological liquid samples such as cell culture media, plasma, serum, urine and other biological fluids: they 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. In general we recommend using a spin filter (**ab93349**) rather than using acid precipitation to remove proteins as acid can deamidate proteins leading to higher ammonia background levels.

For unknown samples, we suggest testing several different doses of samples to make sure the readings are within the standard curve range.

2. Standard Curve Preparation:

Dilute 10 µl of Ammonium Standard II/Ammonium Chloride Standard solution 100 mM in 990 µl of ddH_2O to make a 1 mM Ammonium Standard II/Ammonium Chloride Standard.

Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 100 µl/well with ddH_2O to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Standard II/Ammonium Chloride Standard.

3. Reaction:

Add 80 µl of Reagent II/Reagent 1 to each standard and sample well.
Add 40 µl of Reagent III/Reagent 2 to each well.
Incubate at 37°C for 30 min.

NOTE: 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.

4. Measure OD at 670 nm in a microplate reader.

7. Data Analysis

Correct background by subtracting the value derived from the zero Ammonium Standard II/Ammonium Chloride Standard from all readings. The background reading can be significant and must be subtracted.

Plot the Ammonium Standard II/Ammonium Chloride Standard curve.

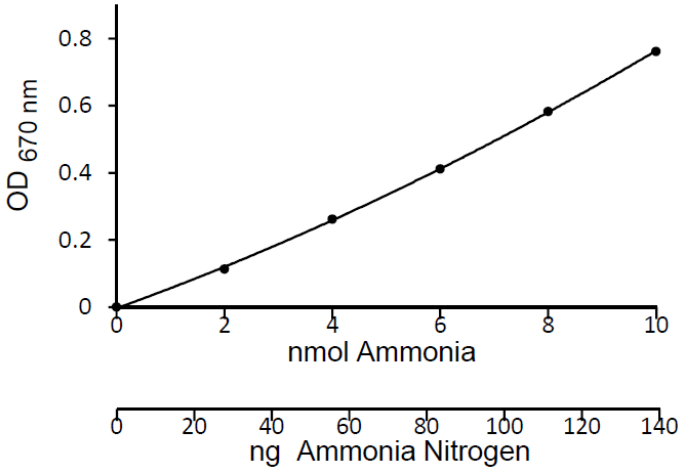
Sample ammonia/ammonium concentrations can then be calculated:

Concentration = Sa / Sv (nmol/µl or mM)

Where:
Sa is the sample amount (in nmol) from standard curve

Sv is the sample volume (µl) added into the wells

NH_4^+ Molecular Weight is 18.04 g/mol



Standard curve generated in standard flat-bottom 96 well plates

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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