Ammonia Colorimetric Assay Kit

Item No. 702400

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Amount	Storage
400669	Ammonia Assay Buffer (10X)	1 vial/1.5 ml	RT
400670	Ammonia Assay Standard	1 vial/500 µl	RT
401128	Ammonia Reagent 1	1 vial/6 ml	-20°C
401129	Ammonia Reagent 2	1 vial/6 ml	-20°C
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Ammonia Colorimetric Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 9)

INTRODUCTION

Background

Ammonia (NH_3), a product of amino acid metabolism, is converted to urea through the urea cycle and exists as the ammonium ion (NH_4^+) at physiological pH.[1] It is generated *via* the breakdown of glutamine in the intestine and skeletal muscle and subsequently detoxified by the liver by conversion to urea or *via* glutamine catabolism in the kidney where it is then excreted in the urine.[2,3] The urea cycle begins in the mitochondria when carbamoyl phosphate synthase 1 (CPS1) condenses ammonia, bicarbonate, and ATP into carbamoyl phosphate but continues in the cytosol after carbamoyl phosphate is converted to citrulline by ornithine transcarbamylase (OTC).[4,5] High ammonia levels (hyperammonemia) can result from several conditions, including diseases that cause liver dysfunction or inborn errors of metabolism caused by deficiencies in urea cycle enzymes or functional deficiencies in urea cycle substrates.[1,2,4,5] Hyperammonemia is neurotoxic and especially deleterious to neonates and infants.[2] It is characterized by cerebral edema, feeding and breathing disruptions, seizures, hypothermia, neurological posturing, lethargy, and coma.[2,5] The most common enzymatic deficiency that causes hyperammonemia is OTC deficiency.[5] Acute liver failure and chronic liver diseases such as cirrhosis cause increased blood ammonia levels, which leads to hepatic encephalopathy.[6]

About This Assay

Cayman's Ammonia Colorimetric Assay Kit provides a colorimetric method for measuring ammonia in plasma, serum, and urine. In the assay, ammonia is converted into NADH in a series of enzymatic reactions. NADH is then oxidized, resulting in the reduction of the tetrazolium salt substrate WST-8 to a highly colored formazan dye, which absorbs at 450 nm (see Figure 1, below). The amount of formazan is proportional to the amount of ammonia in the sample. This assay has a range of 0-60 µM and a lower limit of detection (LLOD) of 1.3 µM.

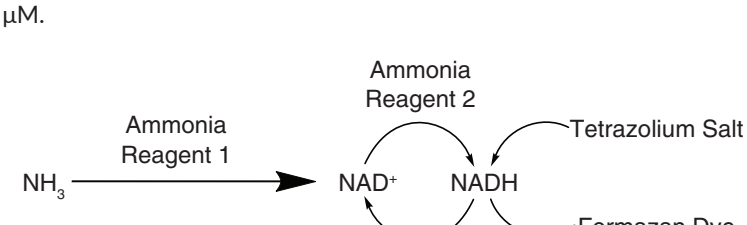

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Ammonia Assay Buffer (10X) - (Item No. 400669)**

   This vial contains 1.5 ml of Ammonia Assay Buffer (10X). To prepare Ammonia Assay Buffer (1X), add 13.5 ml of pure water. The Ammonia Assay Buffer (1X) will be stable for six months when stored at 25°C.

2. **Ammonia Assay Standard - (Item No. 400670)**

   This vial contains 500 µl of 1 mM ammonium chloride in buffer. Dilute 100 µl of the 1 mM Ammonia Assay Standard with 900 µl of Ammonia Assay Buffer (1X) to yield a 100 µM bulk standard. The undiluted standard will be stable for six months when stored at 25°C.

3. **Ammonia Reagent 1 - (Item No. 401128)**

   This vial contains 6 ml of Ammonia Reagent 1 (AR1) and is ready to use as supplied. Thaw AR1 on ice. If not using all at once, prepare aliquots and store at -20°C. Limit freeze-thaw cycles to two.

4. **Ammonia Reagent 2 - (Item No. 401129)**

   This vial contains 6 ml of Ammonia Reagent 2 (AR2) and is ready to use as supplied. Thaw AR2 on ice. If not using all at once, prepare aliquots and store at -20°C. Limit freeze-thaw cycles to two.

Sample Preparation

Ammonia is extremely labile. It is recommended that samples be processed immediately after collection, kept on ice, and assayed the same day. Do not let samples sit on ice for more than 2 hours. If samples cannot be assayed the same day, samples should be flash frozen, stored at -80°C, and assayed within three days.

Plasma

Collect blood in heparin-containing vacutainers. EDTA plasma is not recommended for this assay. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes at 4°C. Transfer the top plasma layer into a clean test tube without disturbing the white buffy layer. In cases where samples are hyperammonemic, it may be necessary to dilute samples 1:2 to 1:5 with Ammonia Assay Buffer (1X) to fall within the range of the standard curve.[7]

Serum

Collect blood in tubes without an anticoagulant. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes at 4°C. Transfer the top serum layer into a clean test tube. In cases where samples are hyperammonemic, it may be necessary to dilute samples 1:2 to 1:5 with Ammonia Assay Buffer (1X) to fall within the range of the standard curve.[7]

Urine

Urine samples should be assayed immediately or stored at -80°C after collection. Interference in urine is infrequent. Dilute urine samples with the Ammonia Assay Buffer (1X) to fall within the range of the standard curve. For human urine, dilutions from 1:500 to 1:4,000 are recommended.[8] Values obtained from urine samples can be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Cayman's Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each sample and standard be assayed at least in duplicate. A typical layout of standards and samples to be measured in duplicate is shown in Figure 2, below. It is suggested that the contents of each well are recorded on the template sheet provided (see page 21).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	1	1	9	9	17	17	25	25	33	33
B	B	B	2	2	10	10	18	18	26	26	34	34
C	C	C	3	3	11	11	19	19	27	27	35	35
D	D	D	4	4	12	12	20	20	28	28	36	36
E	E	E	5	5	13	13	21	21	29	29	37	37
F	F	F	6	6	14	14	22	22	30	30	38	38
G	G	G	7	7	15	15	23	23	31	31	39	39
H	H	H	8	8	16	16	24	24	32	32	40	40

A-H = Standard Wells
1-40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 110 µl in all the wells.
- All reagents should be prepared as described above. The AR1 and AR2 should be kept on ice and all other reagents should be kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate, but it is at the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the absorbance at 450 nm.

Standard Curve Preparation

NOTE: Prior to preparing the standards, ensure that the Ammonia Assay Standard has been diluted as instructed on page 8.

Label eight clean test tubes A-H. Add the amount of bulk ammonia standard (100 µM) and Ammonia Assay Buffer (1X) to each tube as described in Table 1, below. The diluted standards will be stable for four hours at room temperature.

Tube	Ammonia Assay Buffer (1X) (µl)	100 µM Bulk Standard (µl)	Standard (µM)
A	200	0	0
B	190	10	5
C	180	20	10
D	160	40	20
E	140	60	30
F	120	80	40
G	100	100	50
H	80	120	60

Table 1. Preparation of the ammonia standards

Performing the Assay

1. **Standard Wells:** Add 10 µl of standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 2, page 10).
2. **Sample Wells:** Add 10 µl of sample to at least two wells.
3. Initiate the reactions by adding 50 µl of AR1 to all of the wells being used.
4. Incubate for 5 minutes at 37°C.
5. Add 50 µl of AR2 to all wells.
6. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 60 minutes at 37°C.
7. Remove the plate cover and read absorbance at 450 nm.

Procedure	Standard Wells (µl)	Sample Wells (µl)
Add Standard	10	--
Add Sample	--	10
Add AR1	50	50
Incubate for 5 minutes at 37°C		
Add AR2	50	50
Cover and incubate for 60 minutes at 37°C		
Remove cover and read plate at 450 nm		

Table 2. Assay summary

ANALYSIS

Calculations

1. Determine the average absorbance of each standard and sample.
2. Subtract the absorbance value of standard A from itself, all other standards, and all samples to obtain corrected standard or sample measurements (CSM) for each standard and sample.
3. Plot the CSM values of each standard as a function of the final concentration of ammonia from Table 1 on page 12. See Figure 3, on page 15, for a typical standard curve.
4. Calculate the ammonia concentration of the samples using the equation obtained from the linear regression of the standard curve substituting the CSM for each sample.

$$\text{ammonia (µM)} = \left[\frac{\text{CSM - (y-intercept)}}{\text{slope}}\right] \text{x sample dilution}$$

NOTE: To convert to µg/dl, divide the ammonia concentration in µM by 0.587.

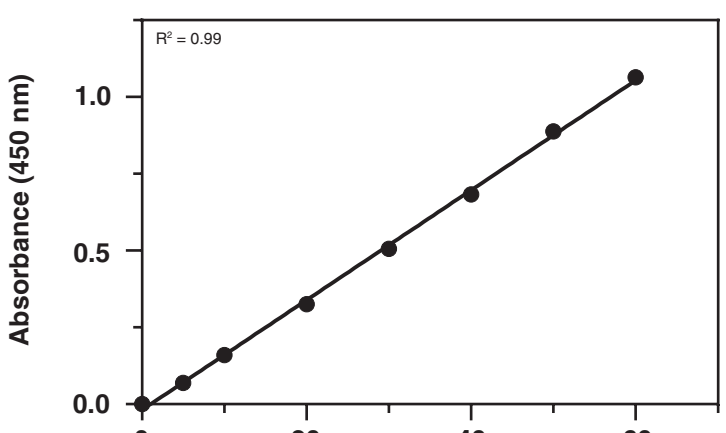

Figure 3. Ammonia standard curve

Performance Characteristics

The assay range describes the lowest and highest concentrations in which ammonia can be reliably detected. The assay range is 0-60 µM.

The lower limit of quantification (LLOQ) is the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.). The LLOQ for the assay is 5 µM.

The lower limit of detection (LLOD) is the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value. The LLOD for the assay is 1.3 µM.

Precision

When a series of 24 plasma, serum, and urine measurements were performed on the same day, the intra-assay coefficients of variation were 3.7, 5.0, and 16.8% respectively. When a series of 5 plasma, serum, and urine measurements were performed on different days under the same experimental conditions, the inter-assay coefficients of variation were 1.0, 4.9, and 10.1% respectively.

Parallelism

To assess parallelism, human plasma (heparin), serum, and urine were serially diluted with Ammonia Assay Buffer (1X), and evaluated using the Ammonia Colorimetric Assay Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

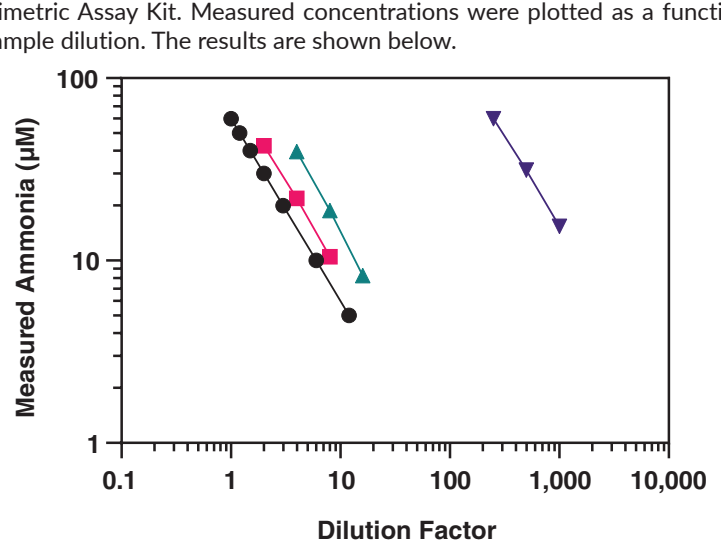

Figure 4. Parallelism of various matrices in the Ammonia Colorimetric Assay

Spike and Recovery

Human plasma (heparin) and serum were spiked with ammonium chloride, serially diluted with Ammonia Assay Buffer (1X), and evaluated using the Ammonia Colorimetric Assay Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

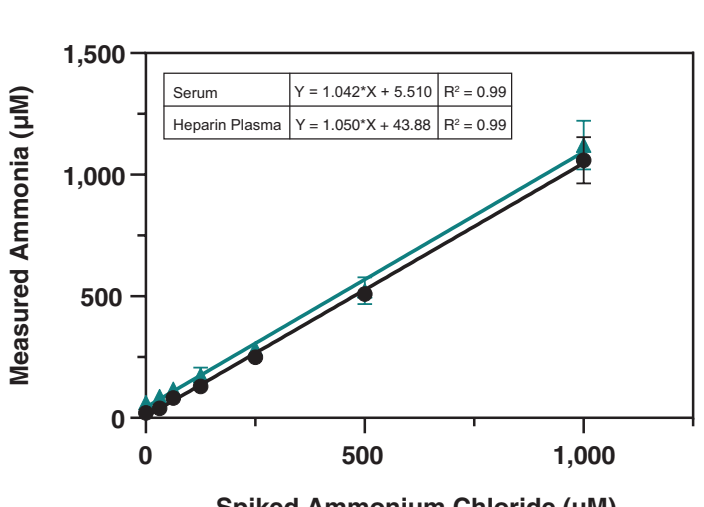

Figure 5. Spike and recovery of ammonia in plasma and serum

Interferences

The following reagents were tested for interference in the assay.

Reagent		Will Interfere (Yes or No)
Buffers	1X PBS, pH 7.4	No
	50 mM HEPES, pH 7.4	No
	50 mM Tris-HCl	No
Detergents	Triton X-100 (≤0.5%)	No
	Tween 20 (≤0.5%)	No
Chelator	EDTA (≤0.1 mM)	Yes
Solvents	DMSO (≤0.5%)	No
	DMF (≤0.5%)	No
	Ethanol (≤0.5%)	No

Table 3. Interferences

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No absorbance detected above background in the sample wells	The target concentration is too low to detect or does not contain target	Re-assay the sample using a lower dilution
Absorbance in the sample wells are above the absorbance of the highest standard	Sample is too concentrated	Re-assay the sample using a higher dilution

References

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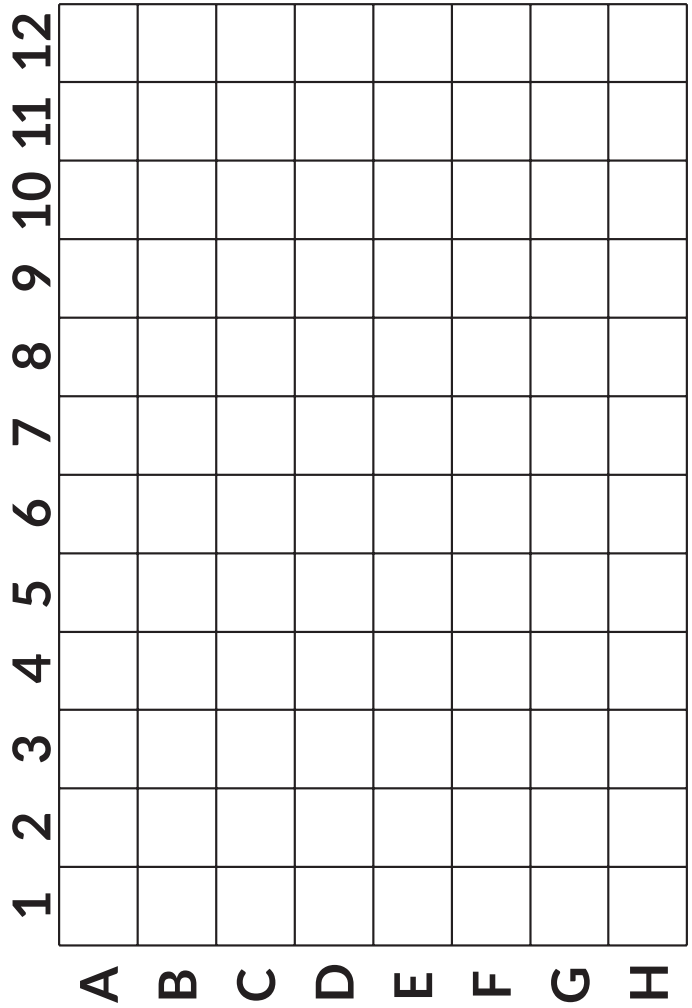
2. Häberle, J. Clinical practice: The management of hyperammonemia. *Eur. J. Pediatr.* **170(1)**, 21-34 (2011).
3. Paulusma, C.C., Lamers, W.H., Broer, S. *et al.* Amino acid metabolism, transport and signalling in the liver revisited. *Biochem. Pharmacol.* **201**, 115074 (2022).
4. Walker, V. Ammonia toxicity and its prevention in inherited defects of the urea cycle. *Diabetes Obes. Metab.* **11(9)**, 823-835 (2009).
5. Summar, M.L. and Mew, N.A. Inborn errors of metabolism with hyperammonemia. Urea cycle defects and related disorders. *Pediatr. Clin. North Am.* **65(2)**, 231-246 (2018).
6. Mallet, M., Desplats, V., Bouzbib, C., *et al.* Blood ammonia in patients with chronic liver diseases: A better defined role in clinical practice. *Anal. Biochem.* **657**, 114873 (2022).
7. Zhu, H., Wan, H., Duan, S., *et al.* Value of monitoring urine ammonia at time of biopsy in patients with lupus nephritis. *BMC Nephrol.* **21**, 442 (2020).
8. Meier, C., Burns, K., Manolikos, C., *et al.* Hyperammonaemia: Review of the pathophysiology, aetiology and investigation. Pathology **56(6)**, 763-772 (2024).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.