AMMONIA
(LIQUID READY™)

VALIDATION REPORT

SKU: 700007403
K-AMIARLQ

11/24

INTRODUCTION:

The Ammonia Assay Kit (Liquid Ready™) is a robust, quick and easy method for the measurement of ammonia in various matrices and is fully automatable for high throughput analysis of samples. Data presented in this report validates that this method is fit for the purpose intended.

RECOMMENDATIONS FOR ANALYSIS:

Please reach out to your local sales representative or to the technical team should you require any assistance, particularly in relation to assay troubleshooting, data analysis, additional matrix testing and application support in relation to automated analyzers.

- This test should only be carried out by trained laboratory personnel. The product instructions must be followed to ensure an accurate and robust result.
- Store the kit at 2 - 8 °C.
- Ensure all kit components come to room temperature 20 - 25 °C before use.
- Use the contents of bottles 1, 2 & 3 as supplied.
- Due to volatility of ammonia, it is recommended to use cuvette caps or parafilm for the manual cuvettes for maximum precision.
- Use a repetitive pipettor to add reagents to the cuvette for increased accuracy.
- The reagent blank value must be determined once for each run and subtracted from each sample result.
- Users should perform matrix validation experiments prior to routine use. This process will highlight any problematic matrices encountered.
- Carrez clarification should not be used in the sample preparation for ammonia determination as it negatively impacts the assay performance.
- Use separate tips for each sample extract and control solutions to avoid cross-contamination. Additionally, pre-flush the tip before pipetting.
- When testing solid samples, ensure a representative portion is taken and homogenized before weighing.

EQUIPMENT (RECOMMENDED):

1. Syringe filters (e.g. AGILENT™ Nylon or equivalent, 0.2 micron); alternatively, eppendorf tubes and centrifuge.
2. Sartorius™ grade 292 filter paper or equivalent.
3. Blender (e.g. Nutribullet™).
4. Analytical balance and spatulas.
5. Wide-mouth bottles (e.g. Duran® bottles, 100 mL and 1 L).
6. Glass graduated cylinders (25 mL, 50 mL) and volumetric flasks (100 mL).
7. 1 M perchloric acid.
8. 8 M potassium hydroxide solution or equivalent.
9. pH-meter or equivalent.
10. Refrigerator or ice bath (or equivalent).
11. Stir plate.

SUMMARY OF PERFORMANCE DATA:

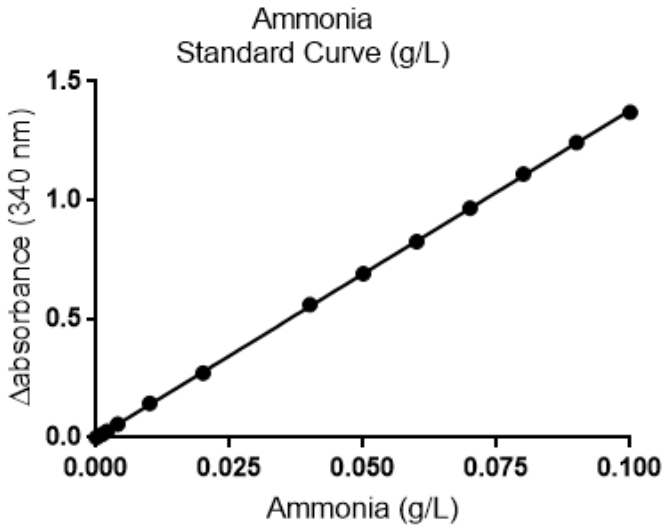
All testing was performed using the standard manual assay described in the product instructions. Results are summarised in the table below:

Recommended working range	0.004 g/L – 0.08 mg/L
Limit of Detection (LOD)	0.001 g/L
Limit of Quantification (LOQ)	0.003 g/L
Limit of Precision	0.004 g/L
Specificity	NH3 - $NH4^+$
Bias %	-2.437%
Acceptable Recovery of Standards	95% - 105%
Stability Studies	24 months shelf life from date of manufacture, see product label for expiry
	Kit performance maintained after 3 freeze-thaw cycles
Robustness	< 5 mins to reach completion at 20^{o}C, 25^{o}C and 37^{o}C
Repeatability	CV < 5% for pure ammonia samples
	CV < 15% for a range of matrices tested
Selectivity/Cross-reactivity	No cross reactivity observed with interfering agents tested
Matrix Interference	Recovery between 95% - 105% in red wine, white wine, apple juice, beef mince and Brie cheese.

LINEARITY AND WORKING RANGE:

The recommended linear measurement range is from 0.004 g/L to 0.01 g/L of ammonia. Samples with concentrations up to 0.1 g/L can be analyzed without dilution, however assay repeatability/robustness may be affected at concentrations above 0.08 g/L. Samples containing higher levels of analyte can be diluted with distilled water before measurement. Values below 0.004 g/L will not fall within the acceptance criteria.

Ammonia (g/L)	$\Delta_{absorbance}$ (340 nm)	Recovery (%)
0	0	0
0.001	0.013	87.9
0.002	0.027	94.3
0.004	0.057	99.9
0.010	0.144	101.5
0.020	0.272	95.50
0.040	0.561	98.57
0.050	0.690	97.02
0.060	0.826	96.81
0.070	0.967	97.14
0.080	1.110	97.48
0.090	1.243	97.10
0.100	1.371	96.36



LIMIT OF DETECTION, QUANTIFICATION AND PRECISION

The LOD is the lowest concentration of the analyte that can be detected by the method. This was determined by testing 20 replicates of the blank (i.e. adding 100 µL of water instead of sample). The $\Delta A_{Limit\ of\ Detection}$ is calculated as 3.3 x s'0; where s'0 is the standard deviation of a number of samples $\Delta_{Absorbance}$ reading.

The LOQ is the lowest level at which the kit's performance is acceptably repeatable. This was determined by testing 20 replicates of the blank (i.e. adding 100 µL of water instead of sample). The $\Delta A_{Limit\ of\ Quantification}$ is calculated as kQ x s'0; where s'0 is the standard deviation of a number of samples $\Delta_{Absorbance}$ reading. The IUPAC default value for kQ is 10. Refer to the product instructions for (g/L) calculation.

The Limit of Precision (g/L) is the analyte concentration at which it was experimentally determined that acceptable recoveries (± 5%) are achieved. It was assessed by measuring 12 replicates of the lowest analyte concentrations, starting at LOQ level.

$\Delta A_{Limit\ of\ Detection}$	Limit of Detection (g/L)	$\Delta A_{Limit\ of\ Quantification}$	Limit of Quantification (g/L)	Precision Limit (g/L)
0.0130	0.001	0.0395	0.003	0.004

NOTE: The above detection limits were calculated based on assay concentration (i.e. samples post-extraction). The dilution used in pre-treatment must be accounted for when establishing the detection limits for specific samples.

TRUENESS AND *BIAS*

The Trueness of the Ammonia Assay kit (Liquid Ready™) was tested using validated aqueous ammonia standards. The *bias* is the comparison of the mean of the results (X) achieved using the standard manual protocol with a suitable reference material.

- Relative Bias is calculated in percent as: $b(\%) = (X - X_{ref}) / X_{ref} \times 100$.

Reference material (g/L)	Replicates, n	% CV	% Recovery	% Bias
0.010	10	1.246	98.02	-2.431
0.050	10	1.170	98.41	-2.160
0.100	10	0.449	99.50	-2.720

Recovery of sample solutions was within the acceptance criteria of 100 ± 5% and %CV below 5%. A negative *bias* is generally observed across all products used for the measurement of Ammonia, due to the volatility of the analyte.

INTERFERENCE AND SELECTIVITY

Selectivity

The assessment of the selectivity of this method in the presence of interfering compounds was tested by spiking a set concentration of interfering agent with a known concentration of validated aqueous ammonia standard and assessing the recovery. Concentrations of interfering agent were selected based on what were considered to be levels likely to be found in relevant samples.

NOTE: Interfering substances in the sample being analyzed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding ammonia to the sample in the initial extraction steps.

Compound tested	Concentration of interferent in assay (g/L)	Recovery of Ammonia (%)
D-Glucose	150	100.19
D-Fructose	80	100.24
D-Galactose	0.5	101.65
Sucrose	200	100.89
Lactose	20	101.95
Glycerol	10	98.35
Sorbitol	200	97.67
DL-Malic acid	5	100.60
DL-Tartaric acid	2.5	100.38
Citric acid	5	97.70
Ascorbic acid	2.5	100.19
Galacturonic acid	10	96.74
L-lactic acid	20	100.82
Acetic acid	1.5	99.87
Ethanol	120	99.52
Sodium Chloride	50	99.94

All recoveries were within the acceptance criteria of 100 ± 5%. No cross-reactivity was found in any of the tested substances.

Matrix Interference

The assessment of the interference of this method when using specific complex matrices was tested by spiking extracted samples with a known concentration of validated aqueous ammonia standard and assessing the recovery.

NOTE: All matrices were prepared according to the sample preparation methods described in the product instruction document which can be found on the product webpage.

Matrix tested	Replicates, n	Ammonia Spike Recovery (%)
Red Wine	2	99.39
White Wine	2	98.59
Apple Juice	2	99.98
Mince Beef	2	99.41
Brie Cheese	2	100.77

Five matrices were tested; all samples fall within 95-105% recovery. Furthermore, the average %CV for all spike recoveries was 1.67% which demonstrates the precision and specificity of this method.

ROBUSTNESS AND STABILITY

Storage Temperature

To assess the storage stability of the test kit components, all kit components were stored at 4°C. Real time monthly performance and enzyme activity testing was conducted, and where relevant slope predictions were used to estimate the product shelf life.

Storage Temperature	Reagent Tested	Stability Data
4°C	Reagent 1	24 months shelf life from date of manufacture, see product label for expiry
	Reagent 2	

The storage robustness of this kit was also tested by performing three freeze-thaw cycles. All kit components were frozen at -20^{o}C overnight and allowed to thaw before testing. This freeze-thaw cycle was repeated 3 times using the same components.

Ammonia Recovery (g/L)				
Expected Ammonia (g/L)	T0	1st Cycle	2nd Cycle	3rd Cycle
0.010	0.010	0.010	0.010	0.010
0.050	0.049	0.050	0.050	0.049
0.100	0.099	0.098	0.100	0.096

Recovery of sample solutions was within the acceptance criteria of 100 ± 5%. ANOVA evaluation of the freeze-thaw cycles shows that there is no significant difference in recovery in assay performance over three consecutive freezing cycles of K-AMIARLQ reagents.

Assay Temperature

Enzymatic test kits are known to be subject to variation when tested under different environmental conditions; temperature being a key parameter that influences reaction rate and analyte recovery. The Ammonia Assay kit (Liquid Ready) was tested using different reaction incubation temperatures (20^{o}C, 25^{o}C and 37^{o}C); recoveries and reaction times were analyzed.

	Ammonia Recovery (g/L)		
Expected Ammonia (g/L)	20°C	25°C	37°C
0.010	0.010	0.010	0.010
0.040	0.041	0.040	0.040
0.100	0.096	0.095	0.095
Reaction Time	3 min	3 min	2 min
Recommended time	*5 minute reaction time is recommended*		

ANOVA evaluation of the effect of temperature in assay shows that there is no significant difference in recovery at the temperatures tested. All recoveries were found to be within acceptance criteria of 100 ± 5% with a faster reaction time observed at 37^{o}C. However, it is recommended to use lower temperature for analysis as ammonia volatility increases with temperature, resulting in reduced precision.

PRECISION AND REPEATABILITY

Precision

Precision is a measure of the variability in results, on different days and by different analysts, over a period of time and using different lots of the test kit. The precision of the Ammonia Assay kit (Liquid Ready™), was investigated using aqueous ammonia samples

Ammonia (g/L)	Replicates, n	Mean (g/L)	Standard Deviation	%CV
0.010	24	0.010	0.0001	0.796
0.040	24	0.040	0.0003	0.846
0.100	24	0.095	0.0005	0.472

All recoveries were shown to be within acceptance criteria of 100 ± 5% and %CV below 5%. This demonstrates that the method is precise and repeatable when using a validated aqueous ammonia standard.

NOTE: Users should perform matrix validation work prior to routine use. This process will highlight any problematic matrices encountered. If you have questions about these or other matrices, please contact your local sales representative for support.

Matrix Repeatability

Matrix repeatability was tested by one analyst over three days using a range of selected matrices. Sample preparation was performed daily to assess precision of analyte extraction as well as precision of the test method. All extractions were performed as per the sample preparation method stated in the product instruction.

Matrix	Replicates, n	Mean result (g/L)	Standard Deviation	%CV
Red wine	6	0.004	0.0026	7.19
White wine	6	0.015	0.0003	2.27
Apple juice	6	0.008	0.0007	8.71
Mince	6	0.011	0.0016	14.47
Brie	6	0.030	0.0020	6.80

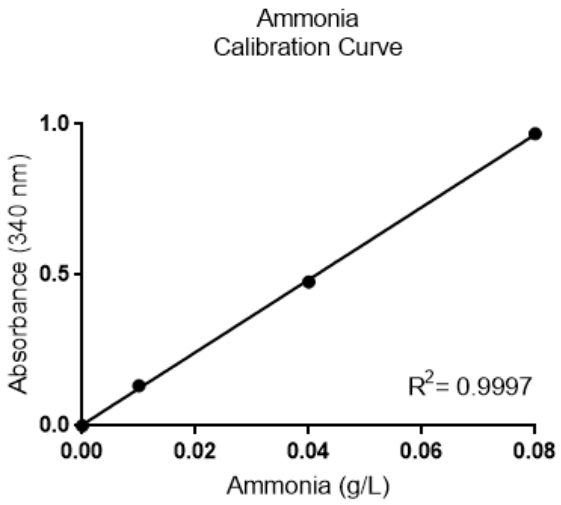
All sample analysis resulted in %CV values of <15 %. Very low levels of naturally occurring ammonia in matrices still resulted in good estimation for precision of extraction. Ammonia is a spoilage marker for mince beef which explains the higher deviation in measurements, as the ammonia levels were increasing gradually with time. The analysis demonstrates that the method is precise and repeatable when testing complex matrices as well as using a validated aqueous ammonia standard.

METHOD AUTOMATION

The Ammonia Liquid Ready™ kit was designed for biochemistry analyzers. It is easily adapted to any instrument. Ammonia content can be determined in a single test using a cubic spline fit.

NOTE: For each batch of samples that is applied to the determination of ammonia a calibration curve must be performed concurrently using the same batch of reagents.

Ammonia (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	%CV
0.01	4	0.0100	0.0019	1.452
0.04	4	0.0399	0.0008	0.174
0.08	4	0.0795	0.0034	0.352



SERVICES AND TECHNICAL SUPPORT

Please reach out to your local sales representative should you require any assistance, particularly in relation to:

- Troubleshooting
- Data analysis
- Additional matrix testing
- Application support in relation to automated analyzers.

Supporting documents can be found in the product page:

- Product Instructions
- Ammonia Liquid Ready™ Quick Reference Guide
- Mega-Calc™ Ammonia Liquid Ready™
- Safety data sheets (SDS)
- Certificate of analysis (CoA)

Megazyme by NEOGEN

Contact us for more information: neogen.com/contact

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