TARTARIC ACID
(LIQUID READY™)

VALIDATION REPORT

SKU: 700007893
K-TARTLQ

11/26

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INTRODUCTION:

The Tartaric Acid Assay Kit (Liquid Ready) is designed to be a robust, quick and easy method for the measurement of Tartaric Acid in various matrices and 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 employees. The product instructions must be followed to help 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.
- Use of repetitive pipettor is recommended to reduce the risk of pipetting error.
- The reagent blank value must be determined once for each set of determinations and subtracted from each sample result.
- The calibrator value must be determined for each set of determinations and used to calculate the Tartaric Acid content of each sample result.
- Users should perform matrix validation experiments prior to routine use. This process will highlight any problematic matrices encountered.
- Use separate pipette tips for each sample extract and control solutions to reduce the risk of cross-contamination. Additionally, pre-flush the tip before pipetting.
- When testing solid samples, ensure a representative portion is homogenized before weighing.

EQUIPMENT (RECOMMENDED):

1. Positive displacement pipettor (e.g. Eppendorf Multipette® with Combitip®) or micro-pipettors (e.g. Gilson® Pipetman®).
2. Macro-cuvettes (e.g. BRAND® PMMA, 1 cm pathlength).
3. Cuvette stoppers/caps or sealing film (e.g. Parafilm™).
4. Heat block incubator (or equivalent) for cuvettes (not required if ambient temperature is in the range of 20-25°C).
5. Spectrophotometer capable of reading at 510 nm.
6. Syringe filters (0.2 micron, e.g. AGILENT® Nylon or equivalent) or Filter paper (e.g. Sartorius® grade 292 or equivalent).
7. Glass Test Tubes (e.g. Pyrex® 16 mm x 100 mm).

SUMMARY OF PERFORMANCE DATA:

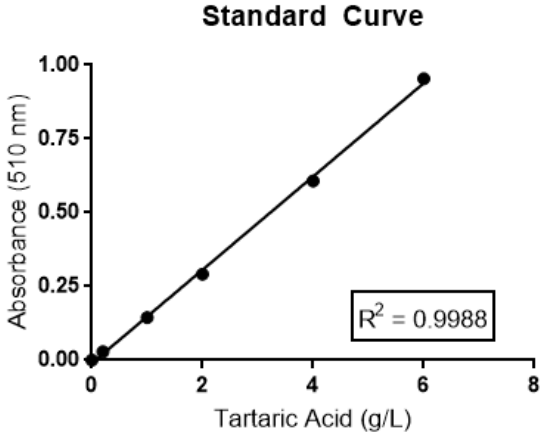
All testing was performed using the standard manual assay described in the product instructions. Results are summarized in the table below:

Recommended working range	0.2 g/L – 6 g/L
Limit of Detection (LOD)	0.051 g/L
Limit of Quantification (LOQ)	0.154 g/L
Limit of Precision	0.2 g/L
Specificity	Tartaric Acid
Bias %	- 3.6 % to 2.4%
Acceptable Recovery of calibrator	90 % - 110 %
Stability Studies	24 months shelf life from date of manufacture, see product label for expiry
Robustness	< 3 mins to reach completion at 20^{o}C, 25^{o}C and 37^{o}C. *NOTE: Manual format is recommended from 20^{o}C to 25^{o}C only*
	Full recovery after 3 freeze-thaw cycles
Verified Matrices	Red Wine, White Wine, Grape Juice and lemon-lime flavoured carbonated beverage
Ruggedness	CV < 5 % for aqueous samples
	CV < 5 % for the range of matrices tested (red wine, white wine, grape juice and lemon-lime flavoured carbonated beverage.)
Cross-reactivity	Fructose > 100 g/L, Ascorbic acid > 0.1 g/L, DL-Lactic Acid > 1 g/L, L-Malic Acid > 1 g/L, Citric Acid > 1.5 g/L.
Matrix Interference	Recovery between 95 % - 105 % in red wine, white wine, sprite and grape juice.

LINEARITY AND WORKING RANGE:

The recommended linear measurement range for Tartaric Acid is 0.2 g/L to 6 g/L. Samples containing analyte concentrations above this range should be appropriately diluted with distilled water prior to analysis to ensure accurate quantification. Samples containing less than 0.2 g/L will fall outside the validated range and will not meet the acceptance criteria.

Tartaric Acid (g/L)	$\Delta_{absorbance}$ (510 nm)	Recovery (%)
0.000	0.000	-
0.200	0.029	96.125
1.000	0.145	95.799
2.000	0.291	95.986
4.000	0.607	98.822
6.000	0.954	103.573



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 21 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 the blank $\Delta_{Absorbance}$ 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 the blank $\Delta_{Absorbance}$ reading. The IUPAC default value for kQ is 10.

The Limit of Precision refers to the lowest analyte concentration at which acceptable recoveries (± 5%) are consistently achieved under routine conditions. This parameter was determined experimentally by analyzing increasing concentrations of the analyte, beginning at the limit of quantification (LOQ). The assessment focused on identifying the lowest concentration level that still met the predefined recovery criteria.

$\Delta A_{Limit\ of\ Detection}$	Limit of Detection (g/L)	$\Delta A_{Limit\ of\ Quantification}$	Limit of Quantification (g/L)	Precision Limit (g/L)
0.012	0.051	0.037	0.154	0.200

NOTE: The above detection limits were calculated based in-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 Tartaric Acid Assay Kit (Liquid Ready) was evaluated using validated aqueous standards. Trueness was assessed by comparing the mean result (X), obtained through the standard manual protocol, with a suitable reference material.

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

Assay Temperature (°C)	Expected (g/L)	Replicates, n	Concentration (g/L)	Standard Deviation	CV (%)	Recovery (%)	*Bias (%)*
20°C	0.2	10	0.194	0.002	1.151	97.09	-2.91
	3.0	10	2.908	0.011	0.371	96.92	-3.08
	6.0	10	6.144	0.029	0.467	102.40	2.40
25°C	0.2	10	0.194	0.004	1.804	97.20	-2.80
	3.0	10	2.892	0.007	0.251	96.39	-3.61
	6.0	10	6.076	0.042	0.685	101.27	1.27

The recovery of the sample solutions fell within the acceptance criteria of 100 ± 5 %, with a coefficient of variation (%CV) below 5 %. The calculated bias for the method ranged from -3.61 % to 2.40 %, indicating acceptable agreement using the reference standard.

INTERFERENCE AND SELECTIVITY:

Interference

The method was evaluated in the presence of potential interfering compounds. This was achieved by spiking a fixed concentration of each interfering agent into a validated aqueous standard containing a known concentration of tartaric acid and assessing the recovery.

NOTE: Interfering substances in the sample being analysed can be identified by including a certified reference material. Quantitative recovery would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding tartaric acid to the sample in the initial extraction steps.

Compound tested	Concentration in assay (g/L)	Recovery of Tartaric Acid (%)
D-Glucose	300	96.8
D-Fructose	300	90.7
	100	95.7
D-Galactose	5	96.9
Sucrose	200	96.5
Lactose	5	97.1
Sorbitol	10	100.2
Mannitol	10	97
Citric Acid	50	0
	1.5	95.3
L-Malic Acid	50	0
	1	95.9
Galacturonic Acid	10	95.6
Ascorbic Acid	10	0
	0.1	96.2
Sodium Chloride	10	96.7
Calcium Chloride	10	98.9
Sulfites	0.2	99.7
Succinic Acid	5	97.4
Ethanol	120	96.5

Glycerol	20	96.6
Gluconic Acid	1	95.4
D/L-Lactic Acid	10	81.1
	1	95.2
D-Mannose	5	96.8

* Red font indicates interference.

The concentrations of the interfering agents were selected to reflect levels likely to be encountered in relevant sample matrices. The data suggests interference with five compounds: D-Fructose (> 100 g/L), Citric Acid (> 1.5 g/L), L-Malic Acid (> 1 g/L), Ascorbic Acid (> 0.1 g/L) and D/L-Lactic Acid (> 1 g/L). All other recovery values fell within the acceptance criteria of 100 ± 5 %, indicating that no significant cross-reactivity occurred with any of the other tested substances.

Matrix Interference

Matrix-based interference for this method was assessed by spiking extracted samples from complex matrices with a known concentration of a validated aqueous tartaric acid (3 g/L) standard and measuring 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	Spike Measured in Assay (g/L)	In-matrix Spike Recovery (g/L)	In-matrix Spike Recovery (%)
Red Wine	2.913	2.853	97.957
White Wine	2.887	2.987	103.465
Grape Juice	2.897	2.974	102.799
Lemon-Lime Carbonated Beverage	2.883	3.001	103.221
Mean	2.895	2.954	101.861
Standard deviation	0.012	0.060	2.266
CV (%)	0.400	1.996	2.225

A total of four matrices were evaluated, with all relevant samples demonstrating recoveries within the acceptable range of 95–105 %. Additionally, variability was low, with % CV values of 2.23 % for recovery and 0.40 % for measured spike concentration, indicating good method precision across different sample matrices.

ROBUSTNESS AND STABILITY

Storage Temperature

To evaluate the storage stability of the test kit components, all materials were stored at 4°C. Real-time performance was conducted on a monthly basis. Slope-based trend analysis was employed to predict the shelf life of the product.

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	
	Calibrator	

The storage robustness of the kit was further evaluated through three freeze-thaw cycles. All kit components were

frozen at -20°C overnight and allowed to thaw before testing. This process was repeated three times using the same reagents.

	Tartaric Acid Recovery (g/L)			
Expected Tartaric Acid (g/L)	T0	1st Cycle	2nd Cycle	3rd Cycle
0.20	0.182	0.190	0.199	0.206
3.00	2.878	2.884	2.978	2.947
6.00	5.766	6.022	6.213	6.084

Reagent performance remained stable across three freeze–thaw cycles at all concentrations tested. Recoveries in each cycle were consistent with the T0 values, and the single-factor ANOVA showed no statistically significant differences between cycles ($F = 0.0020$, $F_{crit} = 4.0662$, $p = 0.999$). These results demonstrate that the reagent is stable through at least three freeze–thaw cycles.

Assay Temperature

Chemical test kits can be sensitive to environmental conditions, with temperature being a key factor influencing reaction rate and analyte recovery. The Tartaric Acid Assay Kit (Liquid Ready) was evaluated at three different reaction temperatures: 20°C, 25°C, and 37°C. Recovery rates and reaction times were analysed across these conditions.

	Tartaric Acid Recovery (g/L)		
Expected Tartaric Acid (g/L)	20°C	25°C	37°C
0.200	0.202	0.199	0.187
3.000	2.987	3.011	2.893
6.000	6.209	6.138	6.114
Reaction Time	3 min	3 min	3 min
Recommended time	*3 minute reaction time is recommended*		

* Red font indicates recovery outside of acceptance criteria

Across the three Tartaric Acid concentrations tested (0.2, 3.0, and 6.0 g/L), recoveries obtained at 20 °C were highly consistent with the corresponding 25 °C measurements while the results obtained at 37C were not within the 95-105% acceptance range at the low-end of the test (93.7% at 0.2 g/L of tartaric acid).

Two-sample t-tests confirmed that temperature-related differences were not statistically significant. For the 20 °C vs 25 °C comparison, the t-statistic ($t = 0.58$, $df = 2$) was well below the critical value ($t_{crit} = 2.92$), with a p-value of 0.31. Similarly, the 37 °C vs 25 °C comparison yielded a non-significant result ($t = 2.58$, $df = 2$, $p = 0.06$. While results at 37 °C were not statistically different from 20 °C ($p = 0.06$), a clear trend toward lower recovery and increased variability was observed. Based on overall performance, precision, and robustness, an operating reaction temperature range of 20–25 °C is recommended for the assay.

PRECISION AND REPEATABILITY

Precision

Precision reflects the consistency of results obtained under varying conditions, including different days, analysts, and reagent lots. The precision of the Tartaric Acid Assay Kit (Liquid Ready) was evaluated using validated aqueous standards.

Tartaric Acid (g/L)	Replicates, n	Mean (g/L)	Standard Deviation	% CV
0.200	24	0.200	0.0048	2.42
3.000	24	2.931	0.0528	1.80
6.000	24	5.944	0.2017	3.39

All recoveries met the acceptance criteria of 100 ± 5%, with coefficients of variation (% CV) consistently below 5 %. These results demonstrate that the method is both precise and repeatable under typical laboratory conditions.

Matrix Repeatability

Matrix repeatability was assessed by two analysts over two consecutive days using a range of selected complex matrices. Sample preparation was conducted daily to evaluate both the precision of analyte extraction and the repeatability of the assay method. All extractions followed the sample preparation protocol outlined in the product instructions.

Matrix	Replicates, n	Mean result (g/L)	Standard Deviation	% CV
Red wine	12	1.965	0.079	4.00
White wine	12	2.003	0.039	1.96
Grape juice	12	0.204	0.007	3.43
Lemon-Lime Carbonated beverage	12	2.048	0.033	1.61

Across all matrices tested, the coefficient of variation (% CV) was consistently below 5 %, demonstrating that the method is precise and repeatable when applied to multiple sample types.

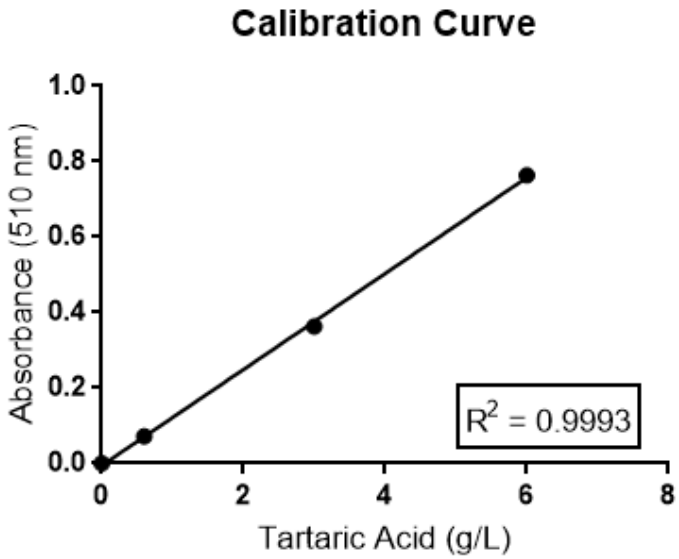
NOTE: Users should perform matrix validation work prior to routine use. This process will highlight any problematic matrices encountered. If you have questions about these or other matrices, please contact your local sales representative for support.

METHOD AUTOMATION

The Tartaric Acid Assay Kit (Liquid Ready) is specifically designed for use with biochemistry analyzers and can be readily adapted to a wide range of instrumentation. Quantification of Tartaric Acid is achieved through a single-test format using a linear calibration fit. The performance data presented below were generated using a ChemWell-T™ analyzer operating at 37°C.

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

Tartaric Acid (g/L)	Replicates, n	Mean result (g/L)	Standard Deviation	% CV
0.60	3	0.597	0.028	4.70
3.00	3	2.997	0.069	2.31
6.00	3	5.995	0.023	0.38



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
- Quick Reference Guide
- Mega-Calc™
- Safety data sheets (SDS)
- Certificate of analysis (CoA)

Contact us for more information: neogen.com/contact

Without guarantee

The information contained in this assay protocol is, to the best of our knowledge, true and accurate, but since the conditions of use are beyond our control, no warranty is given or is implied in respect of any recommendation or suggestions which may be made or that any use will not infringe any patents.

User Responsibility:

- Users are responsible for familiarizing themselves with product instructions and information. Visit our website at neogen.com, or contact your local Neogen® representative or authorized distributor for more information.
- When selecting a test method, it is important to recognize that external factors such as sampling methods, testing protocols, sample preparation, handling, laboratory technique and the sample itself may influence results.
- It is the user's responsibility in selecting any test method or product to evaluate a sufficient number of samples with the appropriate matrices and challenges to satisfy the user that the chosen test method meets the user's criteria.
- It is also the user's responsibility to determine that any test methods and results meet its customers' and suppliers' requirements.
- As with any test method, results obtained do not constitute a guarantee of the quality of the matrices or processes tested.

Terms and Conditions:

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