UV assay for the determination of D-gluconic acid in foodstuffs and other sample materials
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C

This test was evaluated using selected samples of the following matrices: red and white wine, fruit juices and fermented soft drinks.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user

1. Test principle

Enzymatic UV determination of D-gluconic acid and D-glucono-delta-lactone with gluconate kinase (GK), ADP-dependent hexokinase (ADP-HK) and glucose-6-phosphate-dehydrogenase (G6P-DH).

D-gluconic acid + ATP —GK→ D-gluconate-6-phosphate + ADP

ADP + D-glucose — ADP-HK → D-glucose-6-phosphate + AMP

D-glucose-6-phosphate + NAD^+ —G6P-DH→
D-glucono-delta-lacton-6-phosphate + NADH + H^+

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of D-gluconic acid converted and is measured at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, NAD
- Reagent 2: 2 x 12.5 mL with buffer, ADP-HK, G6P-DH, GK

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C / 36 - 46 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 - 8 °C (36 - 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

- Manual and automated sample preparation is the same.
- Samples must be at room temperature for testing.
- Clear and nearly neutral liquid samples may be used directly or after dilution with distilled water to a concentration within the measuring range (see performance data) in the test.
- Cloudy samples must be filtered or centrifugated.
- If necessary, decolorize strongly colored samples.
- Degas carbonated samples.
- For clarification of protein containing samples, Carrez clarification is recommended as described in the following subsections.
  The following chemicals, among others, are required for this:
  1. **Diluted** Carrez solutions:
     - Carrez I: 36 g/L potassium hexacyanoferrate (II) trihydrate
     - Carrez II: 72 g/L zinc sulfate heptahydrate
  2. **Concentrated** Carrez solutions:
     - Carrez I: 150 g/L potassium hexacyanoferrate (II) trihydrate
     - Carrez II: 300 g/L zinc sulfate heptahydrate
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 minutes in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.
- Adjust samples with low pH value using 5 M sodium hydroxide solution to a pH value of 10 - 11.

4. Manual assay procedure

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 6 - 1500 mg/L

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 10 min at 20 - 37 °C and read absorbance A_2.		

4.1. Important notes for assay procedure

- The reagent blank value (water sample) **must be determined once for each run** and subtracted from each sample result.
- Specified incubation times were verified and established at **37 °C (99 °F).** The test may generally perform within a range between **20 - 37 °C (68 - 99 °F).**
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents 1 and reagent 2. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance.
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 2- or 5-minute intervals, for example, until a constant absorbance value is reached.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of D-gluconic acid

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix.

$$C_{\text{D-gluconic acid}}\ [\text{g/L}] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = 0.809 \times \Delta A$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 196.16
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.2. Calculation of solid samples

$$\text{Content}_{\text{D-gluconic acid}}\ [\text{g/100 g}] = \frac{C_{\text{D-gluconic acid}}\ [\text{g/L sample solution}]}{\text{weight}_{\text{sample}}\ \text{in g/L sample solution}} \times 100$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-Acid Standard low (E8460).
The recovery of Enzytec™ *Liquid* Multi-Acid Standard low and other aqueous control solutions should be within 100 ± 5 %.

6. Performance data

6.1. Specificity

The test is specific to D-gluconic acid.

6.2. Interferences & side activities

In the presence of D-/L-malic acid as well as D-/L-tartaric acid and meso-tartaric acid, no interferences could be determined up to 6.25 g/L. Sulfite showed no interference at or below a concentration of 0.5 g/L.
The test shows no side activities to different relevant acids.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2500 mg/L D-gluconic acid, with the recommended measuring range between 6 and 1500 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 1.5 mg/L. With a sample volume of v = 1000 µL, the LoD is 0.25 mg/L.
The limit of quantification (LoQ) was determined by precision profile and is 6 mg/L (100 µL sample volume).
Based on an absorbance difference ΔA = 0.010 and a sample volume of 1000 µL, an LoQ of 1.09 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	60 mg/L
Basic Range	12.5 mg/L
Sensitive Range	2 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	to 9375 mg/L
Basic Range	to 1875 mg/L
Sensitive Range	to 187.5 mg/L

6.4.3. Precision and accuracy

Data from the measurement of an aqueous solution are shown here.

High Range

Target concentration, mg/L	500	1200
Mean value, mg/L	483	1161
SD, mg/L	5.60	6.70
RSD, %	1.16	0.58
Recovery, %	96.5	96.7

Basic Range

Target concentration, mg/L	500	1200
Mean value, mg/L	479.9	1172
SD, mg/L	2.47	3.85
RSD, %	0.51	0.33
Recovery, %	96.0	97.6

Sensitive Range

Target concentration, mg/L	50	120
Mean value, mg/L	48.9	124.7
SD, mg/L	0.26	0.43
RSD, %	0.54	0.35
Recovery, %	96.0	97.6

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid D-Gluconic acid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid D-Gluconic acid* Excel templates for results
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:
https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

- Customized troubleshooting
- Workflow analysis
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

10. Disclaimer

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