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

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) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- For clarification of protein-containing samples, preparation with perchloric acid or trichloroacetic acid is recommended.
- 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 min 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. Assays performance

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.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

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.

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

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

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

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* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

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

9. Disclaimer

This information corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.