Enzymatic assay for the determination of D-gluconic acid in foodstuff and other sample materials
2 x 50 mL R1 and 2 x 12.5 mL R2 – 50 assays (manual) / ≥ 500 assays (auto-analyzer)

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

Method

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

Principle

D-gluconic acid is converted by ATP in the presence of the enzyme GK to D-gluconate-6-phosphate:

D-gluconic acid + ATP $\xrightarrow{\text{GK}}$ D-gluconate-6-phosphate + ADP

The resulting ADP is converted with D-glucose by an ADP-HK to D-glucose-6-phosphate:

ADP + D-glucose $\xrightarrow{\text{ADP-HK}}$ D-glucose-6-phosphate + AMP

D-glucose-6-phosphate + NAD^+ $\xrightarrow{\text{G6P-DH}}$ D-glucono-δ-lactone-6-phosphate + NADH + H^+

Nicotinamide adenine dinucleotide (NAD) is reduced to NADH. The NAD consumption is equivalent to the amount of D-gluconic acid and is measured at a wavelength of 340 nm.

Reagents

The reagents are ready-to-use.

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

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C (see label). Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

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 material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Adjust samples with 5 M NaOH to a pH of 10 – 11.
- Use liquid and clear samples directly or after dilution into the relevant measuring range (see test performance).
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Crush and homogenize solid and semi-solid samples, extract suitable sample amount with perchloric acid and KOH.
- For clarification of protein-containing samples, preparation with perchloric acid or trichloroacetic acid is recommended.
- If necessary, decolorize strongly colored samples with PVPP.
- Detailed sample preparation guideline available on request.

Assay procedure

Wavelength: 340 nm
Temperature: 37 °C or 20 - 25 °C
Measurement: against air or against water
Sample: 2 - 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 or at 20 - 37 °C. Read absorbance A_1, then add:		
Reagent 2	500 µL	500 µL
Mix, wait for the end of the reaction (incubate for about 10 min at 37 °C 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.

Calculation of results

Calculation of sample solutions:

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

df: dilution factor
RB: Reagent blank

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

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

V:	Test volume [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)

For a determination at 340 nm this results in:

$$\mathbf{c_{D\text{-gluconic acid}}\,[g/L] = 0.809 \times \Delta A}$$

Calculation of solid samples:

$$Content_{D\text{-gluconic acid}}\,[g/100\,g] = \frac{c_{D\text{-gluconic acid}}\,[g/L]}{weight_{sample}\,[g/L]} \times 100$$

Performance data

Specificity

The test is specific for D-gluconic acid and shows no side activities or interferences with other relevant acids. In the presence of D-/L-malic acid as well as D-, L- 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 0.75 g/L.

Linearity & Measuring range

Linearity is given up to 2000 mg/L D-gluconic acid. The recommended measuring range is between 2 and 1500 mg/L D-gluconic acid.
If this range is exceeded, the samples should be diluted with dist. water to a gluconic acid concentration within the measuring range.
The dilution factor must be taken into account in the calculation.

Sensitivity

The limit of detection (LoD) and the limit of quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution:

- Sample volume v = 100 µL: LoD = 1.44 mg/L, LoQ = 2.49 mg/L
- Sample volume v = 1000 µL: LoD = 0.25 mg/L, LoQ = 0.42 mg/L

Automation & Validation reports

Application sheets for automated systems and customer validation reports are available on request.

Disclaimer

The data 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.