UV method for approx. 32 assays

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

The method is contained in the German and Swiss food laws. Recommended e.g. by ALVA. Standardized by DIN, ISO, GOST.

Principle

D-Gluconate + ATP ——— Gluconate kinase ⟶ 6-PG + ADP
6-PG + $NADP^+$ ——— 6-PGDH ⟶ ribulose-5-P + NADPH + H^+ + CO_2
D-Glucono-δ-lactone ——— ph 10 ⟶ D-Gluconate

Ref.: Möllering, H. & Bergmeyer, H.U. (1967) Enzymatische Bestimmung von D-Gluconsäure in Lebensmitteln, Z. Lebensm. Unters. Forsch. 135, 198 204

Assay performance

Wavelength:	340 nm (NADPH) ε = 6.3 l x $mmol^{-1}$ x cm^{-1}
Light path:	1.00 cm (glass or plastic cuvettes)
Temperature:	+20 to +25°C
Assay volume:	3.040 ml
Measurement:	against air or against water
Sample solution:	1 to 120 µg D-gluconic acid / D-glucono-δ-lactone in 0.100 to 2.000 ml sample solution.

Reagents

1: Powder mixture with triethanolamine buffer, pH approx. 7.6, approx. 80 mg NADP, approx. 190 mg ATP, magnesium sulfate (for stability see pack label). *Dissolve contents of bottle # 1 with 31 ml redist. water.* The solution is stable for 1 month at +2 to +8°C, resp. 2 months at -15 to -25°C.

2: Approx. 0.7 ml 6-phosphogluconate dehydrogenase (6-PGDH) suspension (approx. 160 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

3: Approx. 0.7 ml gluconate kinase suspension (approx. 18 U) in ammonium sulphate (for stability see pack label). The suspension is ready for use. Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit):

Standard solution sodium or potassium D-gluconate, 0.6 g D-gluconic acid/l for test control only.

The reagents for the determination of D-gluconic acid / D-glucono-δ-lactone are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Procedure

Pipette into cuvettes:	**Blank**	**Standard[1]**	**Sample[2]**	**Rerun assay[3]**	**Assay with internal standard[4]**	**High sensitive assay[5]**
Tea buffer, NADP, ATP solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml-	1.000 ml
Sample solution[6] (e.g. 0.06 bis 0.6 g D-gluconic acid/l)	**-**	**-**	**0.100 ml**	**0.200 ml**	**0.100 ml**	**2.000 ml**
Standard solution[6] (e.g. 0.6 g D-gluconic acid/l)	-	0.100 ml	-	-	0.100 ml	-
6-PGDH suspension # 2	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Redist. Water	2.000 ml	1.900 ml	1.900 ml	1.800 ml	1.800 ml	-
Mix[7], after approx. 5 min read the absorbances (A_1). Add:						
Gluconate kinase suspension # 3	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix[7], after approx. 20 min read the absorbances (A_2). Repeat absorbance reading after another 2 min[8].						

Notes

1 Run a „standard“ to see „accidents“ in analysis. The measurement of the standard is not necessary for calculating results.
2 This assay together with the blank is a single determination.
3 In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes. Calculate with the resp. v.
4 Recovery = $[(\Delta A_{sample + standard} - \Delta A_{sample}) / \Delta A_{standard}] \times 100$ [%].
5 Assay recommended in the case of trace level compound analysis, with sample volume increased up to 2.000 ml (0.0005 to 0.06 g/l of D-gluconic acid).
6 Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
7 e.g. with a plastic spatula, or after closing the cuvette with Parafilm® (American Can Co., Greenwich Ct., USA).
8 The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until they increase constantly over 2 min. Extrapolate absorbances to the time of the addition of gluconate kinase (suspension # 3).

Calculation

$\Delta A = (A_2 - A_1)_{sample,\ resp.\ standard} - (A_2 - A_1)_{blank}$

c = (V x MW x Δ A) / (ε x d x v x 1000)

c = (3.040 x 196.1 x Δ A) / (6.3 x 1.00 x 0.100 x 1000) = **0.9463 x Δ A [g D-gluconic acid/l sample solution]**

c = (3.040 x 178.1 x Δ A) / (6.3 x 1.00 x 0.100 x 1000) = **0.8594 x Δ A [g D-glucono-δ-lactone/l sample solution]**

If the sample has been diluted during preparation, multiply the result with dilution factor F.

When analyzing samples which are weighed out for the sample preparation, calculate the content from the amount weighted:

$$Content_{D\text{-gluconic acid/D-glucono-}\delta\text{-lactone}} = \frac{C_{D\text{-gluconic acid/D-glucono-}\delta\text{-lactone}}\ [g/l\ sample\ solution]}{weight_{sample}\ [in\ g/l\ sample\ solution]} \times 100\ [g/100\ g]$$

Sample preparation:

1. Dilute *clear, colourless and almost neutral liquid samples* to get a sample solution with 0.06 to 0.6 g D-gluconate/l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide,* e.g. by filtration, or add $NaHCO_3$ till the solution is slightly alkaline, dilute (see pt. 1).
4. Adjust *acid (esp. slightly coloured) solutions* with KOH or NaOH to approx. pH 8, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colourless samples.
5. Measure *„strongly coloured solutions"* ph adjusted to 8 against a sample blank (set to zero).
6. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples,* extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.
7. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20 °C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter.
8. Hydrolize D-glucono-δ-lactone with KOH (2 M) at ph 10 to 11 (5 to 10 min at +20 to +25°C).
9. Deproteinize samples with perchloric acid.
   - Homogenize a [g] sample (water content w in g/100 g) with b [ml] perchloric acid (1 M), filter. Neutralize [c] ml filtrate with [d] ml KOH (5 M). Store at +4°C for 15 min, filter.
   - Homogenize a [g] sample with b [ml] perchloric acid (1 M). Neutralize with KOH (5 M). Transfer into a 100 ml volumetric flask, fill up to the mark with redist. water (fatty layer is above the mark). Store at +4°C for 15 min, filter.
   - Homogenize a [g] sample (water content w in g/100 g) with b [ml] perchloric acid (1 M). Neutralize with d [ml] KOH (5 M). Store at +4°C for 15 min, filter.

Assay characteristics

1. *Specific*ity: Specific for D-gluconic acid (D-Glucono-δ-lactone is hydrolized under assay conditions within 50 min.) In the analysis of commercial D-gluconates, results of > 100 % have to be expected when the salts contain free acid and the results are calculated with the molecular weight of the respective salt.
2. *Sensitivity:* 0.25 mg/l (Δ A = 0.005; v = 2.000 ml; V = 3.040 ml)
3. *Detection limit*: 0.5 mg/l (Δ A = 0.010; v = 2.000 ml; V = 3.040 ml)
4. *Linearity* 1 µg/assay (v = 2.000 ml; V = 3.040 ml)
   to 120 µg/assay (v = 0.100 ml; V = 3.040 ml)
5. *Precision:* Δ A = +/- 0.005 to 0.010 absorbance units
   CV = approx. 1 to 2 %
   Pork sausage: r = 0.12 g/100 g $s_{(r)}$ = +/- 0.004 g/100 g
   R = 0.014 g/100 g $s_{(R)}$ = +/- 0.005 g/100 g
   Milk: x = 0.362 g/100 g, r = 0.02 g/100 g, R = 0.08 g/100 g
   Feta cheese: x = 2.57 g/100 g, r = 0.15 g/100 g, R = 0.26 g/100 g