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Product Information

D-Gluconate (D-Gluconic Acid) Assay Kit

Catalog Number **MAK279**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

D-Gluconic acid ($C_6H_{12}O_7$) is a mild organic acid that occurs from the oxidation of glucose. It is naturally present in fruit, honey, and wine. When used as a food additive, it helps regulate acidity. It is a strong chelator, binding anions of calcium, iron, aluminum, copper, and other heavy metals. D-Gluconate ($C_6H_{11}O_7$) describes a salt or ester of gluconic acid. Due to its low toxicity, it is widely used in pharmaceutical, food, and other industries.

The D-Gluconate (Gluconic Acid) Assay Kit is a simple and sensitive method for quantifying D-gluconate. In this assay, gluconate is utilized by gluconokinase to form D-gluconate-6-phosphate and ADP producing a coupled enzyme reaction that generates a colorimetric signal (450 nm), proportional to the amount of D-gluconate present. The assay is sensitive to less than 2 μM of D-gluconate in a variety of samples.

The assay kit is suitable for use with animal tissues, wine, and fruit.

Components

The kit is sufficient for 100 assays in 96 well plates.

Gluconate Assay Buffer 25 mL
Catalog Number MAK279A

Gluconate Converter 1 vl
Catalog Number MAK279B

ATP 1 vl
Catalog Number MAK279C

Gluconate Enzyme Mix 1 vl
Catalog Number MAK279D

Gluconate Developer 1 vl
Catalog Number MAK279E

Gluconate Probe 1 vl
Catalog Number MAK279F

Gluconate Standard (100 mM) 1 vl
Catalog Number MAK262G

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- PVPP (polyvinylpolypyrrolidone), (Catalog number 77627, or equivalent)
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Gluconate Assay Buffer – Allow buffer to come to room temperature before use.

ATP and Gluconate Probe - Reconstitute with 220 μL of water. Mix well by pipetting (do not vortex). Store at –20 °C. Use within 2 months of reconstitution.

Gluconate Converter, Gluconate Enzyme Mix, and Gluconate Developer – Reconstitute with 220 μL of Gluconate Assay Buffer. Mix well by pipetting (do not vortex). Aliquot and store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Gluconate Standard - Reconstitute with 100 μL of water to generate a 100 mM (100 nmole/μL) standard solution. Store at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards.

D-Gluconate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM D-Gluconate Standard with 990 µL of water to prepare a 1 mM (1 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM D-Gluconate standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Gluconate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
The colorimetric assay requires 50 µL of sample for each reaction (well).

Liquid samples (slightly colored, with a neutral pH) can be assayed directly.

Tissue (~10 mg) or cells (~1×10^6) can be homogenized on ice in 100 µL of ice cold Gluconate Assay Buffer. Centrifuge the samples at 10,000 × *g* for 5 minutes. Collect the supernatant.

Add 1–50 µL samples into wells of a 96 well plate. Bring samples to a final volume of 50 µL with Gluconate Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

For samples having high background, prepare parallel sample well(s) as sample background control(s).

Cell and tissue lysates may contain enzymes that consume NADH rapidly. Enzymes should be removed by filtering the samples with a 10 kDa MWCO spin filter.

For liquid samples with a strong color, treat with polyvinylpolypyrrolidone (PVPP) to remove the color. Add PVPP to the sample [1% (w/v) final concentration] and mix for 5 minutes at room temperature. Centrifuge at 10,000 × *g* for 5 minutes. Collect the supernatant. For acidic samples (white wine), neutralize the sample at a 1:1 dilution with 0.5 M Tris HCl, pH 8.0.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Samples and Standards	Sample Control
Gluconate Assay Buffer	40 µL	42 µL
Gluconate Probe	2 µL	2 µL
Gluconate Converter	2 µL	–
ATP	2 µL	2 µL
Gluconate Enzyme Mix	2 µL	2 µL
Gluconate Developer	2 µL	2 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard well. If using a sample background control, add 50 µL of Sample Control Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 40 minutes at 37 °C.

4. For colorimetric assays, measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The reagent background for the assay is the value obtained for the 0 (assay blank) D-Gluconate Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate D-Gluconate standards to plot a standard curve.

Notes: A new standard curve must be set up each time the assay is run.

Subtract the Sample Background Control value from the sample readings to obtain the corrected colorimetric measurement. Using the corrected measurement, determine the amount of D-Gluconate present in the sample from the standard curve.

Concentration of D-Gluconate

$$S_a/S_v = C$$

S_a = Amount of D-Gluconate in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of D-Gluconate in sample

Gluconic Acid molecular weight: 196.16 g/mole

Sample Calculation

Amount of D-Gluconate (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of D-Gluconate in sample

$$5.84 \text{ nmole}/50.0 \ \mu\text{L} = 0.117 \text{ nmole}/\mu\text{L}$$

$$0.117 \text{ nmole}/\mu\text{L} \times 196.16 \text{ ng/nmole} = 23.0 \text{ ng}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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