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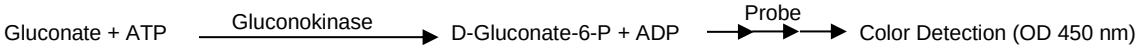
D-Gluconate (D-Gluconic Acid) Assay Kit (Colorimetric)

(Catalog # K683-100; 100 assays; Store at -20°C)

I. Introduction:

D-Gluconic acid ($C_6H_{12}O_7$) is a mild organic acid that is produced from glucose by glucose oxidase. It is abundantly present in plants, fruits and animal tissues. D-Gluconate ($C_6H_{11}O_7$) is the salt or ester of Gluconic Acid. Due to its low toxicity, it is widely used in pharmaceutical, food, and other industries. BioVision's D-Gluconate (Gluconic Acid) Assay kit is a sensitive, fast and easy-to-use kit. In this assay, Gluconate is utilized by Gluconokinase to form D-Gluconate-6-P and ADP, which subsequently undergoes a series of reactions to form an intermediate that reduces Gluconate probe to give a product with strong absorbance at 450 nm. This assay kit can detect D-Gluconate (D-Gluconic Acid) level less than 2 µM in a variety of samples.



II. Application:

- Measurement of D-Gluconate (D-Gluconic Acid) in various samples such as tissues, plants, fruits and wine

III. Sample Type:

- Animal tissues (e.g. muscle etc.)
- Wine (e.g. red wine and white wine)
- Fruits (e.g. orange, apple etc.)

IV. Kit Contents:

Components	K683-100	Cap Code	Part Number
Gluconate Assay Buffer	25 ml	WM	K683-100-1
Gluconate Converter	1 vial	Blue	K683-100-2
ATP	1 vial	Orange	K683-100-3
Gluconate Enzyme Mix	1 vial	Purple	K683-100-4
Gluconate Developer	1 vial	Green	K683-100-5
Gluconate Probe	1 vial	Red	K683-100-6
Gluconate Standard	1 vial	Yellow	K683-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage Conditions and Reagents Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Gluconate Assay Buffer:** Warm to room temperature before use.
- **Gluconate Converter, Gluconate Enzyme Mix, and Gluconate Developer**: Reconstitute each with 220 µl Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **ATP and Gluconate Probe**: Reconstitute with 220 µl dH_2O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.
- **Gluconate Standard:** Reconstitute with 100 µl dH_2O to generate 100 mM (100 nmol/µl) Standard solution. Store at -20°C. Use within two months. Keep on ice while in use.

VII. Assay Protocol:

1. **Sample Preparation:** Liquid samples (slightly colored, pH around neutral) can be measured directly. Tissue (~10 mg) or cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Assay Buffer. Centrifuge at 10,000 x g for 5 min. and collect the supernatant. Add 1-50 µl sample into desired well(s) in a 96 well plate and bring the volume to 50 µl with Assay Buffer.

   Notes:

   a. For unknown samples, we suggest doing pilot experiment and testing several doses to ensure the readings are within the standard curve range.

   b. If samples are suspected to have background such as NADH, prepare parallel sample well as sample background control(s).

   c. Cell or tissue lysates may contain enzymes that consume NADH rapidly. We suggest removing these enzymes from sample by using Deproteinizing Sample Preparation Kit (Cat # K808 or equivalent) before performing the assay.

   d. For liquid samples having strong color, we recommend to use polyvinylpyrrolidone (PVPP) to remove the color. Mix sample with 1% PVPP (w/v) for 5 min. at room temperature. Centrifuge at 10,000 x g for 5 min. and collect the supernatant. For acidic sample (e.g. white wine), neutralize the sample (1:1 dilution) with 0.5 M Tris HCl, pH 8.0.

2. **Standard Curve Preparation:** Dilute D-Gluconate to 1 mM (1 nmol/µl) by adding 10 µl of 100 mM D-Gluconate to 990 µl dH_2O. Mix well. Add 0, 2, 4, 6, 8, and 10 µl of 1 mM D-Gluconate into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well D-Gluconate Standard. Adjust the volume to 50 µl/well with Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
Gluconate Assay Buffer	40 µl	42 µl
Gluconate Converter	2 µl	----
ATP	2 µl	2 µl
Gluconate Enzyme Mix	2 µl	2 µl
Gluconate Developer	2 µl	2 µl
Gluconate Probe	2 µl	2 µl

Mix and add 50 µl of Reaction Mix to each well containing Standard and sample. Mix well.
* Add 50 µl of Background Control Mix to sample background control well.

4. Measurement: Incubate the plate at 37°C for 40 min. Measure absorbance (OD 450 nm).

5. Calculation: Subtract 0 Standard reading from all readings. If sample background control is significant, subtract sample background control reading from sample reading. Plot the Standard Curve. Apply the corrected sample reading to Standard Curve to get B nmole of Gluconate in the sample well.

Sample Gluconate concentration (C) = B/V x Dilution Factor = nmol/µl or µmol/ml or mM

Where: **B** is amount of Gluconate (Gluconic Acid) in the sample well (nmol)
V is sample volume used in the reaction well (µl)

Gluconic Acid molecular weight: 196.16 g/mol.
Gluconic Acid in Sample can also be expressed in nmol/mg of sample.

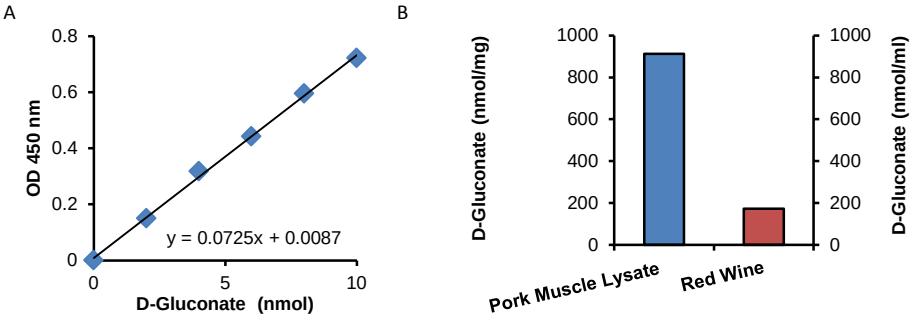


Figure: (A) Gluconate Standard Curve. (B) Measurement of Gluconate in pork muscle lysate (40 µg) and red wine (4 µl). Muscle lysate was deproteinized using Deproteinizing Sample Preparation Kit (Cat # K808) and PVPP was used to decolorize the red wine. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Colorimetric Assay Kit II (K686)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glucose Dehydrogenase Activity Colorimetric Assay Kit (K786)
Glucose-1-Phosphate (G1P) Colorimetric Assay Kit (K697)
Free glycerol Assay kit (K630)
NAD/NADH Quantification Kit (K337)

Glucose Uptake Fluorometric Assay Kit (K666)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay Kit (K788)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)
NADP/NADPH Quantification Kit (K347)

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