ab204703

D-Gluconate Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate measuring of D-Gluconate.

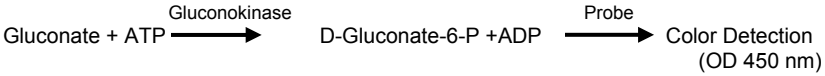
This product is for research use only and is not intended for diagnostic use.

Version 4a, Last Updated 2 June 2025

Table of Contents

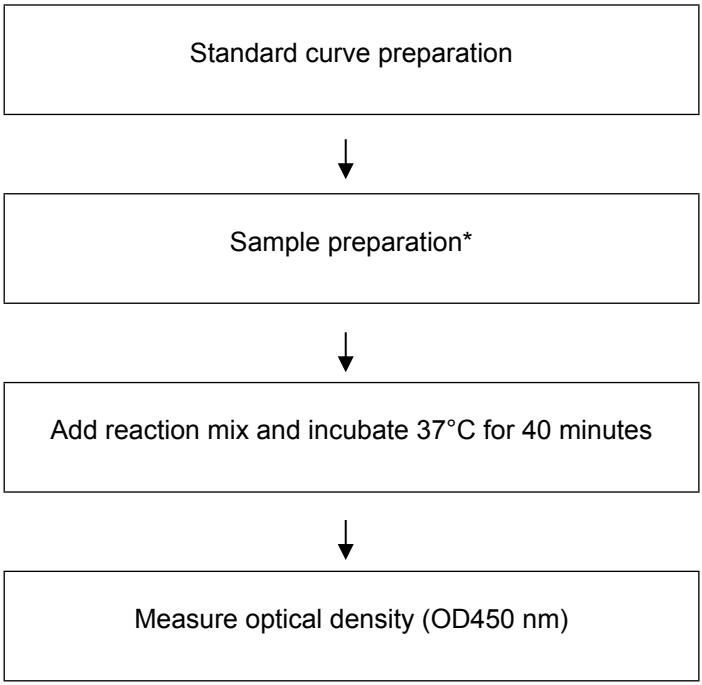
1. BACKGROUND

D-Gluconate Assay Kit (Colorimetric) (ab204703) 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 Developer Solution III to give a product with strong absorbance at OD=450 nm. This assay kit can detect D-Gluconate (D-Gluconic Acid) level less than 2 µM in a variety of samples.



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.

2. ASSAY SUMMARY



*Samples might require deproteinization.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 22	25 mL	-20°C	-20°C
Gluconate Converter Mix	1 vial	-20°C	-20°C
ATP II	1 vial	-20°C	-20°C
Enzyme Mix XVI	1 vial	-20°C	-20°C
Developer Mix G	1 vial	-20°C	-20°C
Developer Solution III	1 vial	-20°C	-20°C
Gluconate Standard	1 vial	-20°C	-20°C

PLEASE NOTE: ATP II was previously labelled as ATP, and Enzyme Mix XVI as Gluconate Enzyme Mix, and Developer Solution III as Gluconate Probe, and Assay Buffer 22 as Assay Buffer XXII and Gluconate Assay Buffer , and Gluconate Converter Mix as Gluconate Converter, and Developer Mix G as Development Enzyme Mix IX and Gluconate Developer. The composition has not changed.

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plate with flat bottom
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

- Deproteinizing Sample Preparation Kit – TCA (ab204708): for deproteinization step
- Polyvinylpyrrolidone (PVPP) - for liquid samples with strong color
- (Optional) 0.5M Tris HCl, pH 8.0 – to neutralize acidic samples

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 22:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Gluconate Standard:**

Reconstitute the Gluconate Standard in 100 µL of ddH_2O to generate a 100 mM (100 nmol/µL) standard stock solution. Pipet up and down to completely dissolve. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.3 **Gluconate Converter Mix:**

Reconstitute in 220 µL Assay Buffer 22. Pipet up and down to completely dissolve. Aliquot converter so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.4 **ATP II:**

Reconstitute in 220 µL ddH_2O. Pipet up and down to completely dissolve. Equilibrate to room temperature before use. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

9.5 **Enzyme Mix XVI:**

Reconstitute in 220 µL Assay Buffer 22. Pipet up and down to completely dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.6 **Developer Mix G:**

Reconstitute in 220 µL Assay Buffer 22. Pipet up and down to completely dissolve. Aliquot developer so that you have enough volume to perform the desired number of assays.

Store at -20°C. Keep on ice while in use. Use within two months.

9.7 **Developer Solution III:**

Reconstitute in 220 µL ddH_2O. Pipet up and down to completely dissolve. Equilibrate to room temperature before use. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the Developer Solution III/probe is thawed, use within two months.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Prepare a 1 mM (1 nmol/µL) Gluconate standard by diluting 10 µL of the reconstituted 100 mM Gluconate standard with 990 µL of ddH_2O.

10.2 Using 1 mM Gluconate standard, prepare standard curve dilution as described in the table in a microplate or in microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 22 (µL)	Final volume standard in well (µL)	End Conc. Gluconate in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer 22.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge sample at 10,000 x *g* for 5 minutes at 4°C using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.1.8 Cell samples may contain enzymes that consume NADH rapidly. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in section 11.4.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of ice cold Assay Buffer 22.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge sample at 10,000 x *g* for 5 minutes at 4°C using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.2.8 Tissue samples may contain enzymes that consume NADH rapidly. Remove these enzymes from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described in Section 11.4.

11.3 **Liquid Samples:**

For liquid samples having strong color, we recommend to use polyvinylpyrrolidone (PVPP) to remove the color.

11.3.1 Mix sample with 1% PVPP (w/v) for 5 minutes at room temperature

11.3.2 Centrifuge at 10,000 x *g* for 5 minutes and collect the supernatant.

For acidic samples (e.g. white wine), neutralize the sample (1:1 dilution) with 0.5 M Tris HCl, pH 8.0.

11.4 **Alternative deproteinization protocol:**

For this step you will need additional reagents:

- Perchloric acid (PCA) 4M, ice cold
- Potassium hydroxide (KOH), 2M

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

11.4.1 Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. ***NOTE:*** *high protein concentration samples might need more PCA.*

11.4.2 Incubate on ice for 5 minutes.

11.4.3 Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.

11.4.4 Precipitate excess PCA by adding ice-cold 2M KOH that equals 34% of the supernatant to your sample (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA.

11.4.5 After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.

11.4.6 Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

11.4.7 **Sample recovery:**

The deproteinized samples will be diluted from the original concentration.

To calculate the dilution factor of your final sample, simply apply the following formula:

$$\%\ original\ concentration = \frac{initial\ sample\ volume}{(initial\ sample\ volume + vol\ PCA + vol\ KOH)}\ x\ 100$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 22).
- Background control sample wells= 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 22).

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 22	**40**	**42**
Gluconate Converter Mix	**2**	**-**
ATP II	**2**	**2**
Enzyme Mix XVI	**2**	**2**
Developer Mix G	**2**	**2**
Developer Solution III	**2**	**2**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 50 µL of appropriate Reaction Mix into each standard and sample wells.

12.4 Add 50 µL of Background Reaction Mix to Background control sample wells.

12.5 Mix and incubate at 37°C for 40 minutes protected from light.

12.6 Measure output at OD = 450 nm on a microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of D-Gluconate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Concentration of Gluconate (nmol/µL or µmol/ml or mM) in the test samples is calculated as:

$$Gluconate = \left(\frac{A}{B}\right) * D$$

Where:

A = Amount of Gluconate (Gluconic Acid) in the sample well calculated from the standard (nmol).

B = Sample volume added into the reaction well (µL).

D = Sample dilution factor.

Gluconic Acid molecular weight = 196.16 g/mol.

Gluconic Acid in Sample can also be expressed in nmol/mg of sample.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

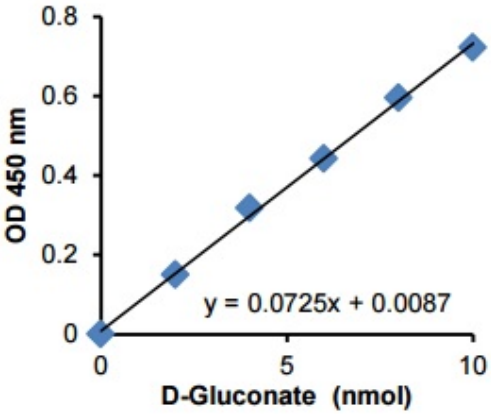


Figure 1. Typical Gluconate Standard calibration curve.

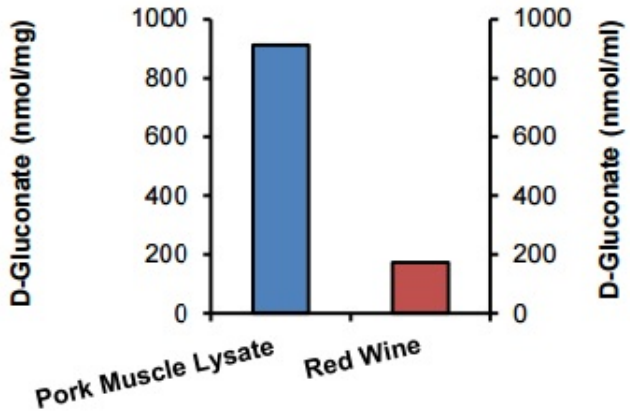


Figure 2. Measurement of Gluconate in pork muscle lysate (40 µg) and red wine (4 µL). Muscle lysate was deproteinized and PVPP was used to decolorize the red wine.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, Developer Solution III, Developer Mix G and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings), including deproteinization step.
- Set up plate for standard (50 µL), samples (50 µL) and background wells (50 µL).
- Prepare Gluconate Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 22	**40**	**42**
Gluconate Converter Mix	**2**	**-**
ATP II	**2**	**2**
Enzyme Mix XVI	**2**	**2**
Developer Mix G	**2**	**2**
Developer Solution III	**2**	**2**

- Add 50 µL of Gluconate Reaction Mix to the standard and sample wells.
- Add 50 µL of Background Control Mix to the background control well.
- Incubate plate at 37°C 40 minutes protected from light.
- Measure plate at OD 450 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use Deproteinizing Sample Kit – TCA (ab204708) or PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

18. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)