Version 2 Last updated 2 June 2023

ab273319 Glyoxalase II Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273319
(use https://www.abcam.cn/ab273319 for china, or
https://www.abcam.co.jp/ab273319 for Japan)

For the determination of Glyoxalase II activity in tissue, cell samples and protein preparations.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Glyoxalase II Activity Assay Kit (GloII) (ab273319) utilizes the ability of an active Glo-II to cleave a substrate while producing D-lactate. The produced D-Lactate reacts with the chromophore generating a stable signal that can be easily quantified at 450 nm using a microplate reader.

Our assay kit is simple, sensitive and can detect as low as 0.6 mU/ml of Glo-II activity in biological samples.

2. Protocol Summary

Prepare Samples, Background Control and Positive Control as directed

Prepare all reagents as directed

Prepare Standards as directed.

Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50 µL

Add Reaction Mix or Background Control Mix (50 µL)

Measure absorbance (OD 405 nm) in kinetic mode for 40 minutes at room temperature.

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 2 month from receipt.

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

Aliquot components in working volumes before storing at the recommended temperature.

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	Quantity	Storage temperature (before prep)
Assay Buffer XII/GloII Assay Buffer	25 mL	-20°C
Enzyme Mix V/Enzyme Mix (Lyophilized)	1 vial	-20°C
Glo II Substrate/GloII Substrate (Lyophilized)	1 vial	-20°C
Developer Solution III/GloII Probe	2 vials	-20°C
Glo II Positive Control/GloII Positive Control	8 µL	-20°C
D-Lactate Standard (100 mM)	100 µL	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 405 nm
- 96-well clear plate with flat bottom
- Ammonium Sulfate Solution (Saturated, 4.1 M)
- Dounce tissue homogenizer

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.
- 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.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer XII/GloII Assay Buffer:**
Bring to room temperature before use.
Store at -20 °C or 4°C.

9.2 **Enzyme Mix V/Enzyme Mix (Lyophilized):**
Reconstitute with 220 µl Assay Buffer XII/GloII Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20 °C. Use within two months.

9.3 **Glo II Substrate/GloII Substrate (Lyophilized):**
Reconstitute with 1.2 mL dH_2O, mix thoroughly. Aliquot and store at -20 °C, protect from light. Use within two months.

9.4 **Developer Solution III/GloII Probe (Lyophilized):**
Reconstitute 1 vial with 220 µl Assay Buffer XII/GloII Assay Buffer and mix thoroughly. Dissolve vial contents when needed. Store at 20 °C. Use within two months.

9.5 **Glo II Positive Control/GloII Positive Control:**
Keep on ice while in use. Store at -20 °C. Use within two months.

9.6 **D-Lactate Standard (100 mM):**
Keep on ice while in use. Store at -20 °C. Use within two months.

10. Sample Preparation

- Endogenous substances from cell or tissue extracts might interfere with the assay generating background. Prepare Sample Background Control well to correct these interferences. Same amount of sample can be tested in the absence of Enzyme Mix V/Enzyme Mix as Sample Background Control. Then, the background reading can be subtracted from the D-lactate reading.
- We suggest using 3-5 different amounts of the samples per well to ensure the readings are within the standard curve range and the changes of velocity are within the lineal range.

Tissue and cell preparation:

10.1 Rapidly homogenize tissue (10-20 mg) or pelleted cells (~1-2 x 10^6) with 300 µl ice-cold Assay Buffer XII/GloII Assay Buffer containing protease inhibitors.
10.2 Keep on ice for 10 mins.
10.3 Centrifuge samples at 12,000 x g at 4°C for 10 minutes.
10.4 Collect the supernatant.
10.5 Remove endogenous interference from tissue and cell samples, as described below:

Ammonium sulfate precipitation method:

10.6 Aliquot samples (2-100 µl) to clean centrifuge tubes, add saturated ammonium sulfate (about 4.1 M at room temperature) to a final concentration 3.2 M.
10.7 Set on ice for 20 minutes.
10.8 Mix well and spin down at 14,000 rpm for 5 mins.

Δ Note: Do not vortex

10.9 Discard the supernatant.
10.10 Repeat the same procedure for one more time and suspend the pellet to the original volume of Assay Buffer XII/Assay Buffer.

Red Blood Cells and Whole Blood:

10.11 Dilute Red Blood Cells (1:100 fold) or Whole Blood (1:50 fold) with ice-cold Assay Buffer XII/GloII Assay Buffer to lyse cells.
10.12 Centrifuge at 6,000 *x g* at 4°C for 10 minutes.
10.13 Collect the supernatant.

Positive Control:

10.14 Prepare a 1:200 dilution of Glo II Positive Control/GloII Positive Control using Assay Buffer XII/GloII Assay Buffer.

11. Standard Curve

Thaw all reagents thoroughly and mix gently.

11.1 Dilute the 100 mM D-Lactate Standard to 1 mM by adding 10 µl of the Standard to 990 µl of Assay Buffer XII/GloII Assay Buffer, mix well.
11.2 Add 0, 2, 4, 6, 8, 10 µl of Diluted D-Lactate Standard into a series of wells and adjust volume to 50 µl/well with Assay Buffer XII/GloII Assay Buffer. This will generate 0, 2, 4, 6, 8, 10 nmol/well of the D-lactate Standard.

Standard #	1 mM D-Lactate Standard (µL)	Assay Buffer XII/GloII Assay Buffer (µL)	D-Lactate (nmol/well)
1	0	50	0
2	2	48	2
3	4	46	4
4	6	44	6
5	8	42	8
6	10	40	10

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1 Add 2-10 µl of sample into desired wells to a 96-well clear plate, labeled as Sample.
12.2 Add the same volume of GloII buffer to wells, labeled as Reagent Background Control.
12.3 Add 4-10 µl of Diluted GloII into Positive Control wells.
12.4 Adjust the volume of Sample, Reagent Background Control and Positive Control wells to 50 µl/well with Assay Buffer XII/GloII Assay Buffer.

Reaction Mix:

12.5 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of appropriate Mix:

Component	Reaction Mix (µL)	Sample Background Control Mix (µL)
Assay Buffer XII/GloII Assay Buffer	34	36
Glo II Substrate/GloII Substrate	10	10
Developer Solution III/GloII Probe	4	4
Enzyme Mix V/Enzyme Mix	2	---

12.6 Mix and add 50 µl of the Reaction Mix to each well containing the D-Lactate Standard, Samples, Reagent Background Control and Positive Control.
12.7 For Background Control, add 50 µl of Sample Background Control Mix to appropriate well and mix well.
12.8 Measure absorbance (OD 405 nm) in kinetic mode for 40 minutes at room temperature.

13. Calculations

13.1 Subtract 0 Standard Reading from all Standard Readings.

13.2 Plot a Standard Curve of OD 450 nm vs. nmol/well D-lactate and obtain the slope of the curve.

13.3 Apply Sample ΔOD and or Sample Background Control ΔOD to D-lactate Standard Curve to obtain the corresponding amounts of D-lactate formed.

13.4 Calculate the Background-Corrected Samples by subtracting sample background control from sample well.

Δ **Note:** If sample background is significant, apply this to correct its corresponding sample.

13.5 Calculate the activity of GloII in the sample as:

$$Sample\ GloII\ activity\ = \frac{B}{(\Delta t\ x\ V)} x\ D \quad (nmol/min/ml)$$

B = D-Lactate from Standard Curve (nmol)
Δt = Reaction time (min)
V = Sample volume added into the reaction well (ml)
D = Dilution factor (D = 1 for undiluted Samples)

Δ **Note:** GloII specific activity can be expressed as U/mg of protein.

Unit definition:
One unit of GloII activity is the amount of enzyme that catalyzes the release of 1 µmol of D-Lactate per min from the substrate under the assay conditions at room temperature.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

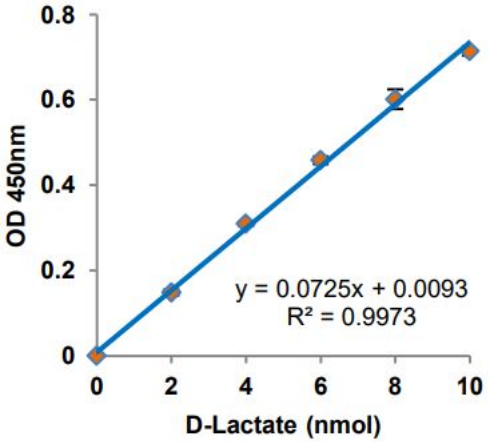

Figure 1. D-Lactate Standard Curve.

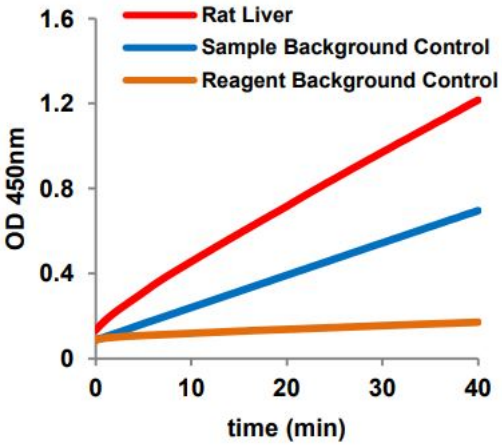

Figure 2. GloII Activity in rat liver tissue extracts (1.5 µg protein).

.

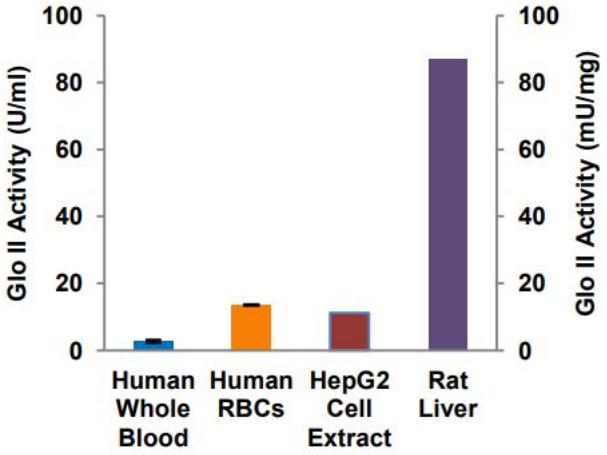

Figure 3. Measurement of GloII activity in human whole blood (6 µl, 1: 50 dilution), human red blood cells (4 µl, 1:100 dilution), HepG2 cell lysates (2 µg protein) and rat liver tissue extracts (1.5 µg protein). All assays were performed following kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. 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)