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ab273284 Methylglyoxal Activity Assay (Fluorometric) Kit

View Kit datasheet: https://www.abcam.com/ab273284
(use https://www.abcam.cn/ab273284 for china, or
https://www.abcam.co.jp/ab273284 for Japan)

For the determination of Methylglyoxal activity in biological samples.

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

Methylglyoxal (MG) Assay Activity Kit (Fluorometric) (ab273284) can detect MG using an enzyme-coupled reaction which reduces the fluorogenic probe. The reduced fluorophore produces a stable signal (Ex/Em= 535/587nm), which is directly proportional to the amount of MG in samples.

The assay is simple, reproducible and can specifically detect as low as 6 pmol of MG in a 100 µl reaction.

2. Protocol Summary

Prepare reagents, Samples and Sample Background Control.

Prepare Standards.

Prepare Reaction Mix and Background Mix and add to appropriate tubes.

Incubate Samples at RT for 60 mins protected from light.

Stop reaction by heating to 95°C for 5 mins. Place Samples on ice protected from light. Spin down.

Transfer 75 µl of each Sample/Background and Controls/Standards to appropriate wells.

Prepare and add to the appropriate wells 25 µl Final Reaction Mix.

Incubate the plate at RT for 2 hrs protected from light.

Measure fluorescence in end-point mode.

Calulate MG concentration using equation.

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.

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)
MG Assay Buffer I/MG Assay Buffer	25 mL	RT
PicoProbe I/Probe (in DMSO)	0.4 mL	-20°C
GSH Standard/Substrate Mix A (Lyophilized)	1 vial	-20°C
Developer XI/Substrate Mix B (Lyophilized)	1 vial	-20°C
Enzyme Mix A	22µL	-20°C
Enzyme Mix B	120 µL	-20°C
Enzyme Mix XIII/Enzyme Mix C (Lyophilized)	1 vial	-20°C
Extraction Solution	2mL	-20°C
Substrate Mix A/MG Standard (20 mM)	1.1 mL	-20°C

7. Materials Required, Not Supplied

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

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96-well white plate with flat bottom
- PCR strip tubes or 0.5/1.5 ml Eppendorf tubes
- 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.

Keep all components on ice during use.

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

9.2 **PicoProbe I/Probe (in DMSO):**
Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.

9.3 **GSH Standard/Substrate Mix A (Lyophilized):**
Reconstitute in 65 µl dH_2O, store at -20 °C. Use within two months

9.4 **Enzyme Mix A and Enzyme Mix B:**
Ready for use, store at -20 °C. Keep on ice when in use.

9.5 **Developer XI/Substrate Mix B (Lyophilized) and Enzyme Mix XIII/Enzyme Mix C (Lyophilized):**
Reconstitute each vial with 220 µl of MG Assay Buffer I/MG Assay Buffer.
Store at -20 °C. Use within two months

9.6 **Extraction Solution:**
Ready to use. Bring to room temperature before use. Corrosive Solution: Make sure to wear gloves and goggles when handling Extraction Solution

9.7 **Substrate Mix A/MG Standard (20mM):**
Ready to use, store at -20 °C. Bring to room temperature before use

10. Sample Preparation

Tissue and Cell Extracts:

10.1 Use 100-200 mg of tissue samples or collect ~10^7 of pelleted cells.

10.2 Mix samples with 180 µl of dH_2O and 20 µl of Extraction Solution. Lyse the bacterial cells with a sonicator.

10.3 Homogenize and vortex, keep on ice for 20 mins.

10.4 Centrifuge samples at 12,000 *x g* at 4 °C for 10 mins.

10.5 Collect the supernatant and dilute it 3-fold with MG Assay Buffer I/MG Assay Buffer to neutralize samples.

Biological Fluids (CSF):

10.6 Deproteinize sample by ultracentrifugation through a 10K Spin Column. Collect the filtrate.

Sample well and Sample Background Control:

10.7 Prepare duplicates by adding 2-40 µl of samples into a PCR strip tubes. Adjust the volume to 50 µl/vial with MG Assay Buffer I/MG Assay Buffer.

Δ Note: MG concentration varies over a wide range depending on the sample. For unknown samples, we recommend doing a pilot experiment and testing several doses to ensure the readings are within the Standard Curve range and the signal kinetics are within the linear range.

Δ Note: To ensure accurate determination of MG in the test samples or for samples having low concentrations of MG, we recommend spiking samples with a known amount of Substrate Mix A/MG Standard (e.g. 20 pmol) and running them in parallel with un-spiked samples.

Δ Note: 0.5/1.5 ml Eppendorf tubes could be used instead of PCR strip tubes.

11. Standard Curve

11.1 Dilute the 20 mM Substrate Mix A/MG Standard to 1 mM by adding 5 µl of the Standard to 95 µl of dH_2O and mix well.

11.2 Further dilute the 1 mM Substrate Mix A/MG Standard to 10 µM (10 pmol/µl) by adding 5 µl of the 1 mM of the Standard to 495 µl of dH_2O.

11.3 Add 0, 2, 4, 6, 8 and 10 pmol/µl Substrate Mix A/MG Standard into a series of vials of PCR strip tubes or Eppendorf tubes.

11.4 Adjust volume to 50 µl/vial with MG Assay Buffer I/MG Assay Buffer to generate 0, 20, 40, 60, 80,100 pmol/well of Substrate Mix A/MG Standards.

Standard #	10 pmol/µl MG-Standard (µL)	MG Assay Buffer I/MG Assay Buffer (µL)	Substrate Mix A/MG Standard (pmol/well)
1	10	40	100
2	8	42	80
3	6	44	60
4	4	46	40
5	2	48	20
6	0	50	0

12. Assay Procedure

Reaction mix:

12.1 In two separate tubes, prepare 10-fold Dilutions of GSH Standard/Substrate Mix A and Enzyme Mix A (i.e. Dilute 2 µl of Substrate/Enzyme Mix A stock solution with 18 µl MG Assay Buffer I/MG Assay Buffer separately). Mix well and keep on ice.

12.2 Prepare enough reagents for the number of assays to be performed:

Component	Reaction Mix (µl)	Background mix (µl)
MG Assay Buffer I/MG Assay Buffer	26	28
Diluted GSH Standard/Substrate Mix A	1	1
Diluted Enzyme Mix A	2	---
Enzyme Mix B	1	1

12.3 Add 30 µl of the Reaction Mix to each vials of PCR strip tubes (or Eppendorf tubes) containing Substrate Mix A/MG Standards, and Sample(s).

12.4 Add 30 µl of Background Mix to vials(s) containing Sample Background Control.

Δ Note: Do not store the Diluted GSH Standard/Substrate Mix A and Diluted Enzyme Mix A. Prepare fresh dilutions as needed.

12.5 Incubate the samples at room temperature for 60 mins avoid light. After incubation time, ensure the cap is securely tightened, and stop the reaction by heating at 95 °C for 5 mins.

12.6 Place samples on ice, avoid light. Spin down.

12.7 Transfer 75 µl of each sample/background controls/standards to desired well(s) to a white, flat-bottom 96-well plate.

12.8 For each well, prepare a total 25 µl Final Reaction Mix containing the following components.

Components	Final Reaction Mix (µl)
MG Assay Buffer I/MG Assay Buffer	20
Developer XI/Substrate Mix B	2
Enzyme Mix XIII/Enzyme Mix C	2
PicoProbe I/Probe	1

12.9 Add 25 µl of the Final Reaction Mix to each well(s) containing the Substrate Mix A/MG Standards, Sample(s) and Sample Background Control.

12.10 Incubate the plate at room temperature for 2 hrs and avoid light. Measure fluorescence at 535/587 nm.

13. Calculations

13.1 Subtract 0 pmol Substrate Mix A/MG Standard reading from all Standards readings.

13.2 Plot the Substrate Mix A/MG Standard Curve.

13.3 Subtract Sample Background Control reading from Sample reading to obtain corrected fluorescence.

13.4 Apply corrected fluorescence to Standard Curve to get B pmol MG in the sample well.

$$MG\ concentration = \frac{B}{V}xD = pmol/\mu l$$

B = MG in sample well based on the Standard Curve slope (pmol)
V = Sample volume added into the reaction (µl)
D = Sample dilution factor (D = 1 for undiluted Samples)

Methylglyoxal molecular weight: 72.06 g/mol

14. Typical Data

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

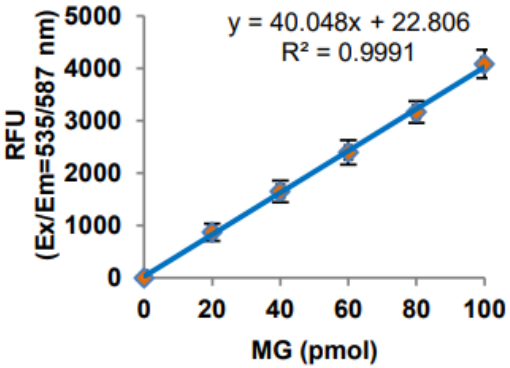

Figure 1. Substrate Mix A/MG Standard Curve, results from multiple experiments.

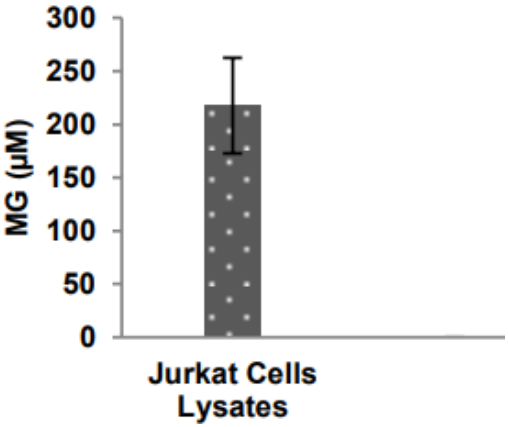

Figure 2. MG activity was measured in Jurkat cells lysate (15 µg lysates). All assays were performed following kit protocol.

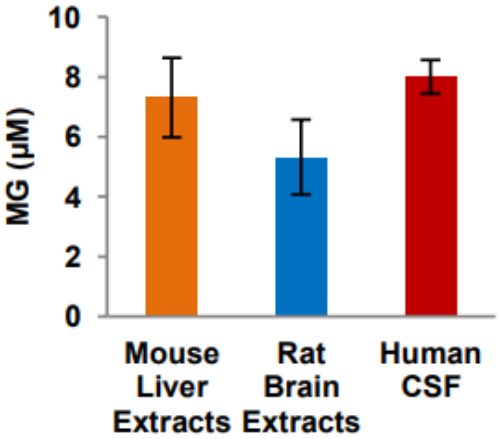

Figure 3. MG activity was measured for mouse liver (4 mg tissue), rat brain (12 mg tissue) and human cerebrospinal fluid (CSF, 2 µl).

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)