Version 3a, Last updated 24 August 2023

ab241006
Methylglyoxal Assay Kit

For the measurement of Methylglyoxal content in food products.

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.

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1. Overview

The Methylglyoxal assay kit (ab241006) enables the detection of MG using a set of engineered enzymes and a chromophore. The reduced chromophore, final product of the assay, produces a stable signal, which can be easily quantified at 450 nm using a microplate reader and its signal is directly proportional to the amount of MG in samples.

The assay is simple, specific, reproducible, and can detect as low as 0.5 nmol/well of MG in a 100 µL reaction.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the standard curve using the 20 mM Methylglyoxal standard. Dilute to 1 mM using dH_20.

Create the Reaction Mix, add 80 µL to each well.

Create the Background Mix and add 80 µL to wells containing sample background control.

Incubate the plate at room temperature for 2 h. Measure absorbance at 450 nm in end-point mode

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Briefly centrifuge small vials prior to opening.

Item	Quantity	Storage condition
MG Assay Buffer II/MG Assay Buffer	25 mL	4°C or -20°C
GSH Standard/Substrate Mix A	1 vial	-20°C
Developer Solution III/Substrate Mix B	1 vial	-20°C
Enzyme Mix A	22 µL	-20°C
Enzyme Mix B	120 µL	-20°C
Enzyme Mix V/Enzyme Mix C	1vial	-20°C
MG Standard/MG Standard (20 mM)	1.1 mL	-20°C

5. 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 absorbance measurement
- 96-well clear plate with flat bottom
- Distilled or deionized water
- Syringe Filter: Pore size 0.22 µm

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 MG Assay Buffer II/MG Assay Buffer:

Store at either 4 °C or -20 °C. Bring to room temperature before use.

6.2 GSH Standard/Substrate Mix A:

Reconstitute in 65 µL dH_2O, store at -20 °C. Use within two months.

6.3 Developer Solution III/Substrate Mix B:

Reconstitute with 220 µL of MG Assay Buffer II/MG Assay Buffer and mix thoroughly. Store at -20 °C.

6.4 Enzyme Mix A:

Ready for use, store at -20 °C, use on ice.

6.5 Enzyme Mix B:

Ready for use, store at -20 °C, use on ice.

6.6 Enzyme Mix V/Enzyme Mix C:

Dissolve in 220 µL MG Assay Buffer II/MG Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20 °C. Use within two months.

6.7 MG Standard:

Store at -20 °C, avoid light. Bring to room temperature before use.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 20 mM MG Standard to 1mM by adding 5 µL of the standard to 95 µL of dH_2O.

7.2 Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM MG standard to wells of the 96 well plate.

7.3 Bring the total volume of each well to 20 µL with dH_2O to generate 0, 2, 4, 6, 8, 10 nmol/well of MG.

Standard #	1 mM MG standard (µL)	dH_2O (µL)	MG concentration Per well (nmol)
1	10	10	10
2	8	12	8
3	6	14	6
4	4	16	4
5	2	18	2
6	0	20	0

8. Sample Preparation

ΔNote: We suggest using 3-5 different amounts of each sample per well to ensure the readings are within the Standard Curve range and the signal kinetics are within the Linear range.

8.1 For liquid samples (i.e. Manuka Honey):

- Manuka Honey (weight: ~200-500 mg) in a centrifuge tube, dilute samples 1:10 (v/v) in dH_2O, mix well.
- Centrifuge samples at 10,000 x g at room temperature for 10 min. Collect the supernatant and filter through 0.22 µM filter.
- Dilute supernatant samples (1:2 to 1:10), if necessary, using dH_2O.
- **Sample(s):** Add 2-20 µL of (diluted) samples onto desired well(s) in a clear 96-well plate.
- **Sample Background Control**: Prepare duplicate sample well(s). Adjust the volume of Sample(s) and Sample Background Controls to 20 µL/well with dH_2O.

  ΔNote: MG concentration varies over a wide range depending on the sample.

9. Assay Procedure

9.1 Prepare a 10-fold Dilution of GSH Standard/Substrate Mix A (i.e. Dilute 2 µL of GSH Standard/Substrate Mix A stock solution with 18 µL MG Assay Buffer II/MG Assay Buffer), mix well and keep on ice.

9.2 prepare a 10-fold Dilution of Enzyme Mix A (i.e. Dilute 2 µL of Enzyme Mix A stock solution with 18 µL MG Assay Buffer II/MG Assay Buffer), mix well and keep on ice.

9.3 Mix enough reagents for the number of assays to be performed. For each well, prepare a total 80 µL Mix containing the following components. Mix well before use:

	Reaction Mix	Background Mix
MG Assay Buffer II/MG Assay Buffer	67 µL	69 µL
Diluted GSH Standard/Substrate Mix A	6 µL	6 µL
Diluted Enzyme Mix A	2 µL	-
Enzyme Mix B	1 µL	1 µL
Enzyme Mix V/Enzyme Mix C	2 µL	2 µL
Developer Solution III/Substrate Mix B	2 µL	2 µL

9.4 Add 80 µL of the Reaction Mix to each well containing the MG Standard(s), Sample(s); Add 80 µL of Background Mix to well(s) containing Sample Background Control.

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

9.5 **Measurement:** Measure absorbance at 450 nm in end-point mode.

10. Data Analysis

10.1 Subtract the 0 MG Standard reading from all Standard curve readings. Plot the background-subtracted MG Standard Curve and calculate the slope.

10.2 If sample background control slope is significant, then subtract sample background control reading from sample readings.

10.3 Apply the corrected ΔRFU value to the MG Standard Curve to get B nmol MG in the sample well.

$$\text{Sample MG Concentration} = (B/V) \times D \text{ nmol}/\mu L = \text{mM}$$

Where:

B is the amount of MG in the sample well from Standard Curve (nmol)
V is the sample volume added into the reaction well (µL)
D is the sample dilution factor

11. Typical Data

Typical data provided **for demonstration purposes only**.

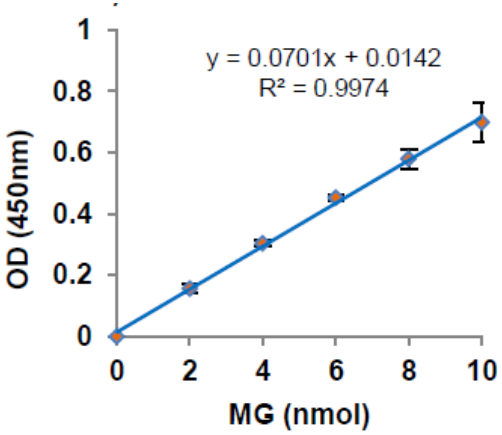

Figure 1. MG Standard Curve.

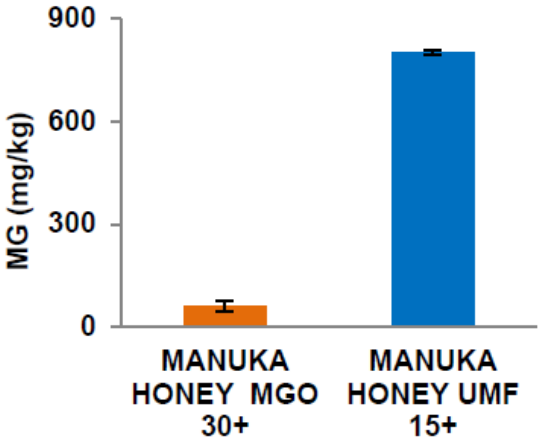

Figure 2. Measurement of MG in Manuka Honey (MGO 30+) (10 µl; Dilution Factor: 2, in dH2O); Manuka Honey (UMF 15+) (10 µl; Dilution Factor: 10, in dH2O). All assays were performed following kit protocols. *According to reference: MG in Manuka Honey (MGO 30+): ≥ 30 mg/kg; MG in Manuka Honey (UMF 15+): ≥ 510 mg/kg.

12. 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)