

Methylglyoxal Assay Kit (Colorimetric) (BN00731)

(Catalog # BN00731; 100 assays; Store at -20°C)

I. Introduction:

Methylglyoxal (MG, 2-oxopropanal, pyruvic aldehyde) is a highly reactive dicarbonyl compound formed during glycolysis and sugar fermentation. It is an important precursor of advanced glycation end products (AGEs). Endogenous MG is present in many food items and beverages. For example, high levels of MG have been reported in Manuka Honey and some soft drinks. Additionally, cooking and some storage techniques can induce the synthesis of MG, such as coffee brewing, and bread toasting. It has been shown that MG has antimicrobial activity against some bacterial strains, including *Streptococcus*, *H. pylori*, *E. coli* and other bacteria resistant to standard antibiotic treatments. Assay Genie's Methylglyoxal Assay Kit (Colorimetric) 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.



II. Applications:

- Measurement of Methylglyoxal in food products

III. Sample Type:

- Food Products: Manuka Honey, etc.

IV. Kit Contents:

| Components | BN00731 | Cap Code | Part Number |
|---------------------|---------|----------|-------------|
| MG Assay Buffer | 25 ml | WM | BN00731-1 |
| Substrate Mix A | 1 vial | Yellow | BN00731-2 |
| Substrate Mix B | 1 vial | Red | BN00731-3 |
| Enzyme Mix A | 22 µl | Violet | BN00731-4 |
| Enzyme Mix B | 120 µl | Orange | BN00731-5 |
| Enzyme Mix C | 1 vial | Green | BN00731-6 |
| MG Standard (20 mM) | 1.1 ml | Brown | BN00731-7 |

V. User Supplied Reagents and Equipment:

- Microplate reader capable of absorbance measurement
- 96-well clear plate with flat bottom
- Distilled or deionized water
- Syringe Filter: Pore size 0.22 µm

VI. Storage Conditions and Reagent Preparation:

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

- **MG Assay Buffer:** Store at either 4 °C or -20 °C. Bring to room temperature before use.
- **Substrate Mix A:** Reconstitute in 65 µl dH₂O, store at -20 °C. Use within two months.
- **Substrate Mix B:** Reconstitute with 220 µl of MG Assay Buffer and mix thoroughly. Store at -20 °C.
- **Enzyme Mix A:** Ready for use, store at -20 °C, use on ice.
- **Enzyme Mix B:** Ready for use, store at -20 °C, use on ice.
- **Enzyme Mix C:** Dissolve in 220 µl MG Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20 °C. Use within two months.
- **MG Standard:** Store at -20 °C, avoid light. Bring to room temperature before use.

VII. Methylglyoxal Assay Protocol:

1. Sample Preparation:

For liquid samples (*i.e.* Manuka Honey): weight (~200-500 mg) Manuka Honey in a centrifuge tube, dilute samples 1:10 (v/v) in dH₂O, 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 dH₂O. **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 dH₂O.

Note:

- 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.
- MG concentration varies over a wide range depending on the sample.
- For samples having high protein content, we recommend deproteinizing the samples using Deproteinizing Sample Preparation Kit II.

2. Standard Curve Preparation: Dilute the 20 mM (20 nmol/μl) MG Standard to 1 mM (nmol/μl) by adding 5 μl of the Standard to 95 μl of dH₂O, mix well. Add 0, 2, 4, 6, 8, 10 μl of 1 mM (nmol/μl) MG Standard into a series of wells. Adjust volume to 20 μl/well with dH₂O to generate 0, 2, 4, 6, 8, 10 nmol/well of MG.

3. Reaction Mix Preparation: Prepare a 10-fold Dilution of Substrate Mix A (i.e. Dilute 2 μl of Substrate Mix A stock solution with 18 μl MG Assay Buffer), mix well and keep on ice; 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), mix well and keep on ice. 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 | 67 μl | 69 μl |
| Diluted Substrate Mix A | 6 μl | 6 μl |
| Diluted Enzyme Mix A | 2 μl | --- |
| Enzyme Mix B | 1 μl | 1 μl |
| Enzyme Mix C | 2 μl | 2 μl |
| Substrate Mix B | 2 μl | 2 μl |

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.

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

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

5. Calculation: Subtract 0 nmol MG Standard reading from all Standard readings. Plot the MG Standard Curve. Subtract Sample Background Control reading from Sample(s) reading to obtain corrected absorbance. Apply corrected absorbance to Standard Curve to get B nmol MG in the sample well.

$$\text{MG concentration in sample (C)} = (B / V) \times D = \text{nmol/}\mu\text{l or mM}$$

Where: **B** is amount of MG in the sample well from Standard Curve (nmol)

V is sample volume added into the reaction well (μl)

D is sample dilution factor

Methylglyoxal molecular weight: 72.06 g/mol.

Methylglyoxal can be expressed as mg/kg.

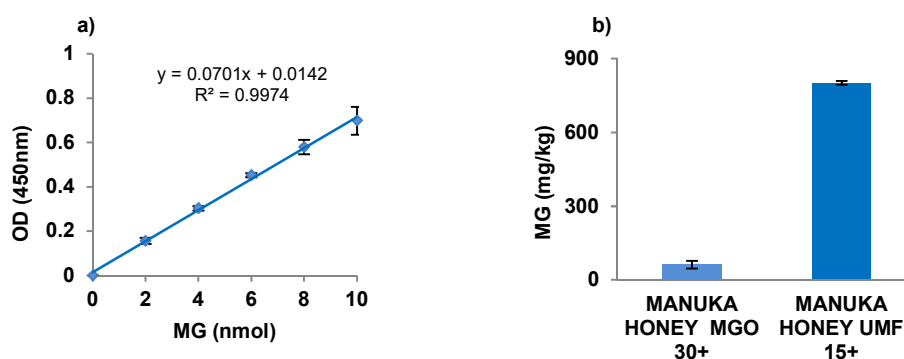


Figure: (a) MG Standard Curve. (b) Measurement of MG in Manuka Honey (MGO 30+) (10 μl; Dilution Factor: 2, in dH₂O); Manuka Honey (UMF 15+) (10 μl; Dilution Factor: 10, in dH₂O). All assays were performed following kit protocols.

*According to reference: MG in Manuka Honey (MGO 30+): ≥ 30 mg/kg; MG in Manuka Honey (UMF 15+): ≥ 510 mg/kg.

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