



- 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 dH<sub>2</sub>O, 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 dH<sub>2</sub>O to generate 0, 2, 4, 6, 8, 10 nmol/well of MG.
- 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.

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

**MG concentration in sample (C) = (B/ V) x D = nmol/μl or mM**

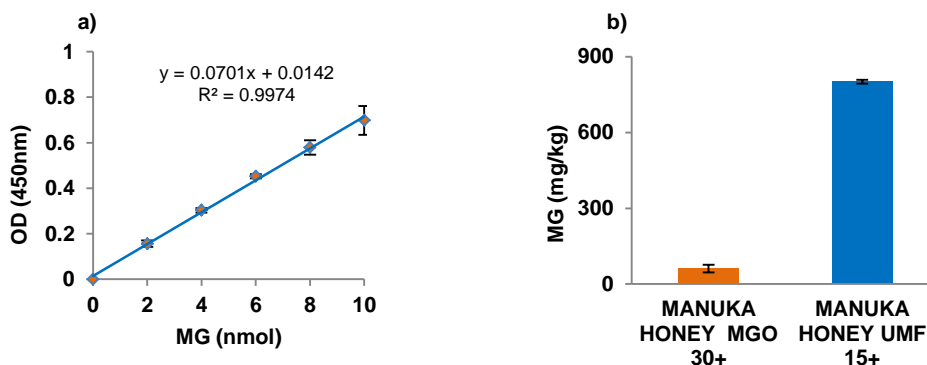
Where: **B** is amount of MG in the sample well from Standard Curve (nmol)

**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 dH<sub>2</sub>O); Manuka Honey (UMF 15+) (10 μl; Dilution Factor: 10, in dH<sub>2</sub>O). 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.

#### VIII. RELATED PRODUCTS:

Glyoxalase I Activity Kit (K591)

Glyoxalase II Activity Kit (K460)

PicoProbe™ Reduced Glutathione (GSH) Assay Kit (K740)

Reduced Glutathione (GSH) Assay Kit (K464)

**FOR RESEARCH USE ONLY! Not to be used on humans**