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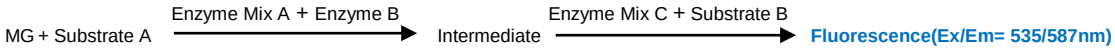
PicoProbe™ Methylglyoxal Assay Kit (Fluorometric)

06/18

(Catalog # K461-100; 100 assays; Store at -20°C)

I. Introduction:

Methylglyoxal (MG, 2-oxopropanal, pyruvic aldehyde), a by-product of glycolysis, is a highly toxic, reactive dicarbonyl compound. It causes non-enzymatic glycation of proteins which yields irreversible advanced glycation end products (AGEs), leading to cross-linking or degradation of proteins. MG is associated with several pathologies including diabetes, aging and neurodegenerative diseases. Furthermore, recent studies have suggested that MG may induce carbonyl stress-type schizophrenia. BioVision's PicoProbe™ Methylglyoxal Assay Kit (Fluorometric) 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.



II. Applications:

- Measurement of Methylglyoxal in various biological samples/preparations

III. Sample Type:

- Tissue Homogenates and Cell Lysates: Mouse Liver, Rat Brain, Jurkat cells, etc.
- Biological Fluids: Cerebrospinal Fluid (CSF), etc.

IV. Kit Contents:

Components	K461-100	Cap Code	Part Number
MG Assay Buffer	25 ml	WM	K461-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K461-100-2
Substrate Mix A	1 vial	Yellow	K461-100-3
Substrate Mix B	1 vial	Red	K461-100-4
Enzyme Mix A	22 µl	Violet	K461-100-5
Enzyme Mix B	120 µl	Orange	K461-100-6
Enzyme Mix C	1 vial	Green	K461-100-7
Extraction Solution	2 ml	Brown/Clear vial	K461-100-8
MG Standard (20 mM)	1.1 ml	Brown/Brown vial	K461-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of fluorescence measurement
- PCR strip tubes
- 96-well white plate with flat bottom
- Dounce Tissue Homogenizer (BioVision: Cat. #1998 or its equivalent)

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 ºC or -20 ºC. Bring to room temperature before use.
- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.
- **Substrate Mix A:** Reconstitute in 65 µl dH_2O, store at -20 °C. Use within two months.
- **Enzyme Mix A and Enzyme Mix B:** Ready for use, store at -20 °C. Keep on ice when in use.
- **Substrate Mix B and Enzyme Mix C:** Reconstitute each vial with 220 µl of MG Assay Buffer. Store at -20 °C. Use within two months.
- **Extraction Solution:** Ready to use. Bring to room temperature before use. **Corrosive Solution:** *Make sure to wear gloves and goggles when handling Extraction Solution.*
- **MG Standard:** Ready to use, store at -20 °C. Bring to room temperature before use.

VII. Methylglyoxal Assay Protocol:

1. Sample Preparation:

Tissue and Cell Extracts: Use 100-200 mg of tissue samples or collect ~10^7 of pelleted cells. Mix samples with 180 µl of dH_2O and 20 µl of Extraction Solution. Homogenize (BioVision, Cat. # 1998) and vortex, keep on ice for 20 min. Centrifuge samples at 12,000 x *g* at 4 °C for 10 min. Collect the supernatant and **dilute it 3-fold with MG Assay Buffer to neutralize samples** (i.e. 100 µl of supernatant with 200 µl of MG Assay Buffer); **Biological Fluids (CSF):** Deproteinize sample by ultracentrifugation through a 10K Spin Column (BioVision, Cat. #1997-25 or its equivalent). Collect the filtrate. **Sample well & Sample Background Control**: 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.

Notes:

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

b. 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 MG Standard (e.g. 20 pmol) and running them in parallel with unspiked samples.

c. 0.5/1.5 ml Eppendorf tubes could be used instead of PCR strip tubes..

2. **Standard Curve Preparation:** Dilute the 20 mM MG Standard to 1 mM by adding 5 µl of the Standard to 95 µl of **dH_2O** and mix well. Further dilute the 1 mM 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**. Add 0, 2, 4, 6, 8, 10 µl of 10 pmol/µl MG Standard into a series of vials of PCR strip tubes or Eppendorf tubes. Adjust volume to 50 µl/vial with MG Assay Buffer to generate 0, 20, 40, 60, 80,100 pmol/well of MG Standards.

3. **Reaction Mix Preparation:**

a. In two separate tubes, prepare 10-fold Dilutions of **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 separately)), mix well and keep on ice. Prepare enough reagents for the number of assays to be performed:

	Reaction Mix	Background Mix
MG Assay Buffer	26 µl	28 µl
Diluted Substrate Mix A	1 µl	1 µl
Diluted Enzyme Mix A	2 µl	---
Enzyme Mix B	1 µl	1 µl

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

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

b. Incubate the samples at room temperature for 60 min, avoid light. After incubation time, ensure the cap is securely tightened, and stop the reaction by heating at 95 °C for 5 min. Place samples on ice, avoid light. Spin down.

c. Transfer 75 µl of each sample/background controls/standards to desired well(s) to a white, flat-bottom 96-well plate. For each well, prepare a total 25 µl Mix containing the following components.

	Reaction Mix
MG Assay Buffer	20 µl
Substrate Mix B	2 µl
Enzyme Mix C	2 µl
PicoProbe™	1 µl

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

4. **Measurement:** Incubate the plate at room temperature for 2 h, avoid light. Measure fluorescence at 535/587 nm in end point mode.

5. **Calculation:** Subtract 0 pmol MG Standard reading from all Standards readings. Plot the MG Standard Curve. Subtract Sample Background Control reading from Sample reading to obtain corrected fluorescence. Apply corrected fluorescence to Standard Curve to get B pmol MG in the sample well.

Sample MG Concentration (C) = B/V X D pmol/µl or µM

Where: B is amount of MG in the sample well from Standard Curve (pmol)
V is sample volume added into the reaction well (µl)
D is sample dilution factor

Note: For spiked samples, correct for any sample interference by using the following equation:

$$\textbf{MG amount in spiked sample (B)} = \left(\frac{(\text{RFU (SAMPLES, corrected)})}{(\text{RFU(SPIKED SAMPLES, corrected)})-(\text{RFU(SAMPLES, corrected)})}\right) * MG\ spike\ (pmol)$$

Methylglyoxal molecular weight: 72.06 g/mol

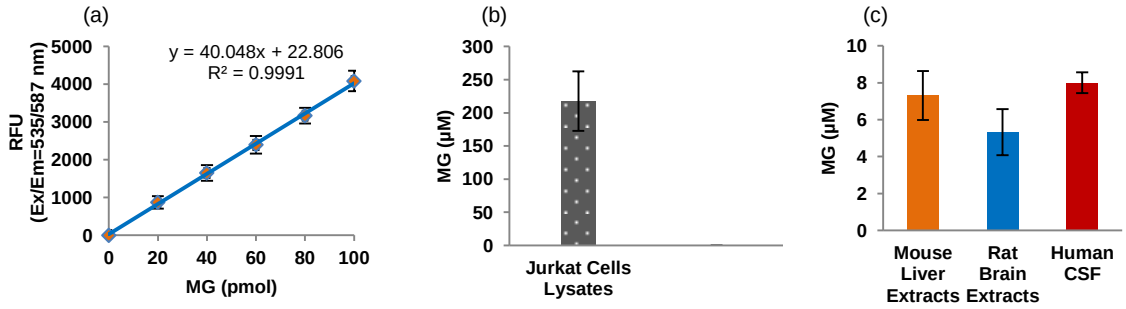


Figure: (a) MG Standard Curve, results from multiple experiments. (b-c) Measurement of MG in Jurkat Cells Lysates (15 µg lysates), Mouse Liver (4 mg tissue), Rat Brain (12 mg tissue) and Human Cerebrospinal Fluid (CSF, 2 µl). All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Methylglyoxal Assay Kit (Colorimetric) (K500)
Glyoxalase I Activity Kit (K591)
Dounce Tissue Homogenizer (1998)
Glyoxalase II Activity Kit (K460)

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