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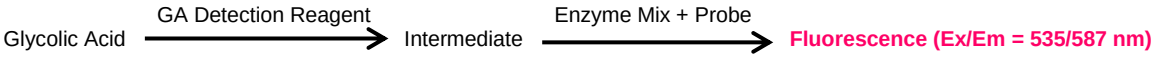
Glycolic Acid Assay Kit (Fluorometric)

10/19

(Catalog # K2022-100; 100 assays; Store at - 20ºC)

I. Introduction:

Glycolic Acid (GA), also known as Hydroxyl Acetic Acid is the smallest alpha hydroxy acid. Although it is used in several skin care products intended for dermal application in small doses, its acute dose can lead to skin and eye irritation. It is nephrotoxic if swallowed and its toxicity is due to its conversion to oxalic acid, which then precipitates with calcium to form crystals leading to renal tissue injury. GA is also a major downstream metabolite of ethylene glycol metabolism, and a concentration higher than 8 mM in serum indicates the need for hemodialysis. Normally serum GA levels lie in the very low micromolar range (roughly 2 µM). **BioVision's Glycolic Acid Assay Kit** is a robust, one step plate based assay for the measurement of GA in serum, urine and plasma samples. The GA Detection Reagent metabolizes GA leading to the formation of an intermediate. The intermediate in the presence of GA enzyme mix then converts the non-fluorescent GA probe to a fluorescent product, which is measured at Ex/Em = 535/587 nm. This kit can quantify as low as 30 pmol of GA.



II. Applications:

Quantification of Glycolic acid

III. Sample Type:

- Serum
- Plasma
- Urine

IV. Kit Contents:

Components	K2022-100	Cap Code	Part Number
GA Assay Buffer	25 ml	WM	K2022-100-1
GA Detection Reagent	220 µl	Blue	K2022-100-2
GA Enzyme Mix	1 vial	Green	K2022-100-3
GA Probe	0.2 ml	Red	K2022-100-4
GA Standard	100 µl	White	K2022-100-5

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- 10 kDa Spin Column (BioVision Cat. # 1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least three months.

- **GA Assay Buffer:** Warm to room temperature (RT) before use.
- **GA Detection Reagent:** Aliquot and store at -20ºC. Avoid repeated freeze-thaw cycles. Keep on ice in use.
- **GA Enzyme Mix**: Reconstitute in 220 µl GA assay buffer. Aliquot and store at -20ºC. Avoid repeated freeze-thaw cycles.
- **GA Probe:** Thaw at RT, protect from light. Store at -20°C.
- **GA Standard (50 mM):** Aliquot and store at -20°C. Thaw at RT before use.

VII. GA Assay Protocol:

1. Sample Preparation:

For **Serum** samples, dilute the sample at 1:10 using GA Assay Buffer before analysis. For **Plasma**, filter the samples through a 10 kDa spin column (BioVision Cat. # 1997) and dilute the ultrafiltrate at 1:10 dilution using GA Assay Buffer. **Note:** For both Serum and Plasma, samples with expected GA concentration more than 5 mM may be diluted to 1:20 dilution. For each Test Sample, add the same Sample volume (1-4 µl) into two parallel wells of a 96-well black plate with flat bottom labeled as **"Sample"** and **"Sample Background Control"**. Adjust the volume of each well to 50 µl/well with GA Assay Buffer.

For **Urine** samples, filter the samples through a 10 kDa spin column (BioVision Cat. # 1997). **Note:** Various compounds present in Urine can interfere in the assay making the use of single point Standard addition with a known amount of GA necessary. For each Test Sample, add the same volume (2-4 µl) of Sample into three parallel wells of a black, flat bottom 96-well plate labeled as '**Sample Background Control**", "**Unspiked Sample**" and "**Spiked Sample**" (containing Sample spiked with 0.4 nmol of GA Standard i.e 4 µl of 100 µM GA Standard solution). Adjust the volume to 50 µl/well with GA Assay Buffer.

Note: We recommend testing several dilutions of the Sample to ensure that the readings are within the linear range of the Standard Curve.

2. Standard Curve Preparation: Prepare 5 mM GA working Standard solution by diluting the provided 50 mM GA Standard solution (1:10 dilution) with water. Further dilute the 5 mM working Standard solution to 100 µM by diluting it at 1:50 with water. Add 0, 2, 4, 6, 8, and 10 µl of the 100 µM GA Standard solution into a 96-well black plate to generate 0.2, 0.4, 0.6, 0.8, 1 nmol/well GA Standard. Adjust the volume of all wells to 50 µl/well with GA Assay Buffer.

Note: If only Urine samples are being run, a Standard Curve is not necessary as single point Standard addition is being done. For Assay Blank, add 50 µl GA Assay Buffer to a well.

3. Reaction Mix Preparation: Mix enough Reaction Mix (for Standard, Assay Blank and Sample wells) and Background Mix (for Sample Background Control wells) according to the table below. Make sufficient amount of each type of the mix to add 50 µl to all assay wells of that type. Mix well.

	Reaction Mix	Background Mix
GA Assay Buffer	44 µl	46 µl
GA Detection Reagent	2 µl	-
GA Enzyme Mix	2 µl	2 µl
GA Probe	2 µl	2 µl

Add 50 µl of Reaction Mix to all the Standard and Sample wells, and 50 µl of Background Mix to the Sample Background Control wells.

Notes:

a) Have the microplate reader ready at Ex/Em = 535/587 nm in a kinetic mode at RT set to record fluorescence every 30 sec.

b) Prepare Reaction Mix immediately before adding to the wells.

4. Measurement: Immediately start recording fluorescence in a kinetic mode at 30 sec intervals for 60-90 min at RT. Readings may also be taken in end point mode after 60 min.

5. Calculation:

Serum/Plasma Sample: Subtract the 0 Standard reading from all Standard readings. Plot the GA Standard Curve. Subtract the Sample Background Control readings from Sample readings. If the 0 Standard readings are higher than Sample Background Control readings, subtract those from Sample readings instead. Calculate the amount of GA in the Sample wells using the following formula**:**

GA concentration in Sample: C = (B / V) X D (nmol/ml or µM)

Where **B** = Amount of GA in the Sample well obtained from the Standard Curve (nmol)
V = Volume of Sample added into the well (ml)
D = Dilution factor (in case Sample was diluted before adding to the reaction well, D = 1 for undiluted Samples).

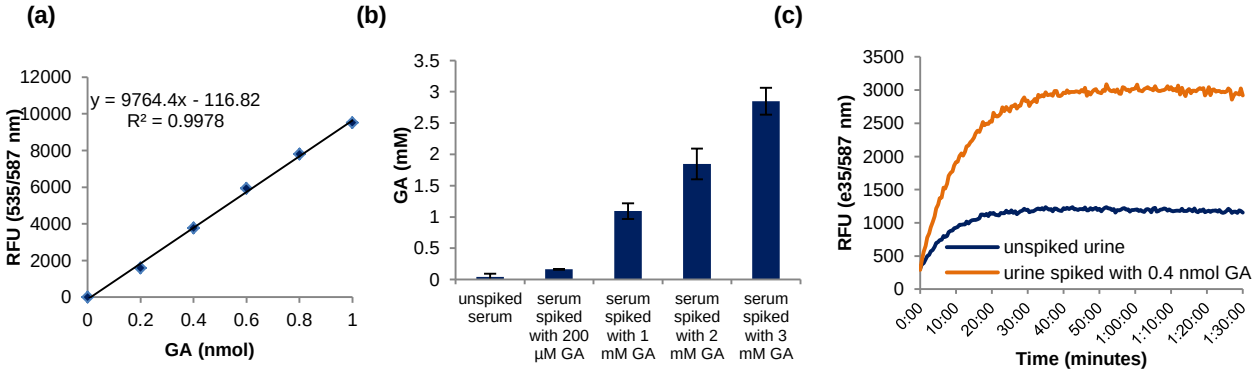
Urine Sample: For both Unspiked and Spiked Sample wells, subtract the Sample Background Control reading from Unspiked Sample and Spiked Sample readings respectively. If "Assay Blank" readings are higher than Sample Background Control readings, then subtract those instead. Calculate the amount of GA in the Unspiked Sample wells using the following formula.

$$\textbf{Amount of GA in Sample wells } (B) = \frac{GA\ (Unspiked)}{GA\ (Spiked) - GA\ (Unspiked)}\ x\ 0.4\ nmol$$

Note: For Samples in which the calculated amount of GA is higher than 1 nmol, the Sample should be diluted further and tested again. Calculate the GA concentration using the following formula**:**

GA concentration in Sample: C = (B / V) X D (nmol/ml or µM)

Where **B** = Amount of GA in the Sample well obtained after single point standard addition
V = Volume of Sample added into the well (ml)
D = Dilution factor (in case Sample was diluted before adding to the reaction well, D = 1 for undiluted Samples).



Figures: (a) GA Standard Curve. **(b)** Estimated GA concentrations in human Serum samples. Spiked experiments show 90±10% recovery. Experiments were performed according to the kit protocol. **(c)** Kinetic data for Urine sample, unspiked, and spiked with 0.4 nmol GA. GA was calculated to be 130 µM.

VIII. Related Products:

Oxalate (Oxalic Acid) Colorimetric Assay Kit (K663)
Hydroxy Acid Oxidase Activity Assay Kit (Fluorometric) (K2010)
Lactate (human) ELISA Kit (E4341)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)

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