Technical Bulletin

Glycolic Acid Assay Kit (Fluorometric)

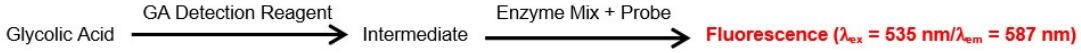
Catalog Number MAK428

Product Description

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 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).

The 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. In the presence of GA enzyme mix, the intermediate quantitatively converts the non-fluorescent GA probe to a fluorescent product, which is measured at λ_{Ex} = 535 nm/λ_{Em} = 587 nm. This method can quantify as little as 30 pmol of GA.

The kit is suitable for the measurement of glycolic acid in serum, plasma, and urine.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- GA Assay Buffer — 25 mL
  Catalog Number MAK428A
- GA Detection Reagent — 220 µL
  Catalog Number MAK428B
- GA Enzyme Mix — 1 vial
  Catalog Number MAK428C
- GA Probe — 200 µL
  Catalog Number MAK428D
- GA Standard — 100 µL
  Catalog Number MAK428E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Black flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

GA Assay Buffer: Warm to room temperature prior to use.

GA Detection Reagent: Aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles. Keep on ice while in use.

GA Enzyme Mix: Reconstitute vial with 220 µL of GA Assay Buffer. Aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.

GA Probe: Thaw at room temperature, protected from light. Store at -20 °C.

GA Standard (50 mM): Aliquot and store at -20 °C. Thaw at room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum

Dilute serum sample 1:10 using GA Assay Buffer before analysis. Samples with expected GA concentrations >5 mM may be diluted 1:20.

Plasma

Filter plasma samples through a 10 kDa spin column such as Corning® Spin-X® UF concentrator. Dilute the ultrafiltrate 1:10 using GA Assay Buffer before analysis. Samples with expected GA concentrations > 5 mM may be diluted 1:20.

Serum or Plasma

For each serum or plasma Sample, add the same volume (1-4 µL) into two parallel wells of a black, flat bottom 96-well plate. Designate as "Sample" (S) and "Sample Background Control" (SBC). Adjust the total volume of each well to 50 µL/well with GA Assay Buffer. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.

Urine

1. Filter urine samples through a 10 kDa spin column such as Corning® Spin-X® UF concentrator. Various compounds present in Urine can interfere in the assay, making the use of a single point Standard addition with a known amount of GA necessary.
2. For each urine Sample, add the same volume (2-4 µL) into three parallel wells of a black, flat bottom 96-well plate. Designate as "Sample Background Control" (SBC), "Sample" (S, Sample without spike), and "Spiked Sample" (Sample + GA Spike; SS).
3. Prepare a 5 mM GA Standard solution by diluting the 50 mM GA Standard 1:10 with purified water.
4. Prepare a 100 µM GA Standard by diluting the 5 mM GA Standard from Step 3 1:50 with purified water.
5. Add 4 µL of 100 µM GA Standard Solution to each Spiked Sample (SS) well.

6. Adjust the total volume of each well to 50 µL with GA Assay Buffer. For unknown samples, test several dilutions to ensure that the readings are within the linear range of the Standard Curve.
7. For Assay Blank for use with Urine samples, add 50 µL of GA Assay Buffer to a well.

Standard Curve Preparation

Note: If only Urine samples are being run, a Standard Curve is not necessary as single point Standard addition is being done

1. Prepare a 5 mM GA standard solution by diluting the 50 mM GA Standard 1:10 in purified water.
2. Prepare a 100 µM GA Standard by diluting the 5 mM GA Standard from Step 1 1:50 with purified water.
3. Prepare GA Standards according to Table 1. Mix well.

Table 1.
Preparation of GA Standards

Well	100 µM GA Standard	GA Assay Buffer	GA (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	0.2
3	4 µL	46 µL	0.4
4	6 µL	44 µL	0.6
5	8 µL	42 µL	0.8
6	10 µL	40 µL	1

4. Set the microplate reader at λ_{Ex} = 535 nm/λ_{Em} = 587 nm in kinetic mode at room temperature set to record fluorescence every 30 seconds.

Reaction Mixes

1. **Immediately** before adding to the wells, mix enough reagents for the number of assays to be performed.
   a. For each well containing Standard, Assay Blank (for urine samples), Sample (S), and Spiked Sample (SS), prepare 50 µL of Reaction Mix according to Table 2. Mix well.
   b. For each Sample Background Control (SBC) well, prepare 50 µL of Background Control Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Control Reaction 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

2. Add 50 µL of the Reaction Mix to each well(s) containing the Standards, Assay Blank, Samples (S), and Spiked Sample (SS). Mix well.
3. Add 50 µL of the Background Control mix to Sample Background Control (SBC) well(s). Mix well.

Measurement

Immediately start recording fluorescence (RFU) at λ_{Ex} = 535 nm/λ_{Em} = 587 nm in kinetic mode at 30 second intervals for 60-90 minutes at room temperature. Readings may also be taken in end point mode after 60 minutes.

Results

Serum/ Plasma Results

1. Subtract the 0 Standard RFU reading from all Standard RFU readings.
2. Plot the GA Standard Curve.
3. Subtract the Sample Background Control (SBC) RFU readings from Sample (S) RFU readings.
4. If the 0 Standard readings are higher than Sample Background Control (SBC) readings, subtract 0 Standard readings from Sample (S) and Spiked Sample (SS) readings instead of the SBC readings.
5. Calculate the amount of GA in the Sample wells using the following formula:

GA concentration (nmol/mL or µM) =

$$(B/V) \times D$$

where:

B = Amount of GA in the Sample well obtained from the Standard Curve (nmol)

V = Volume of sample added to the well (in mL) (1 mL = 1000 µL)

D = Sample dilution factor (for undiluted Samples, D = 1)

Urine Results

1. For both Sample (unspiked, S) and Spiked Sample (SS) wells, subtract the Sample Background Control (SBC) RFU reading from Sample (S) and Spiked Sample (SS) RFU readings respectively.
2. If Assay Blank RFU readings are higher than Sample Background Control (SBC) RFU readings, subtract Assay Blank readings from Sample (S) and Spiked Sample (SS) readings instead of the SBC readings.
3. Calculate the amount of GA in the Sample wells using the following formula:

GA Amount (B) in Sample for Spiked Sample =

$$\left[\frac{\text{Sample RFU}_{\text{Corrected}}}{\text{Spiked Sample RFU}_{\text{Corrected}} - \text{Sample RFU}_{\text{Corrected}}}\right] \times 4 \text{ nmol}$$

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 (nmol/mL or µM) =

$$(B/V) \times D$$

Figure 1.
Typical GA Standard Curve

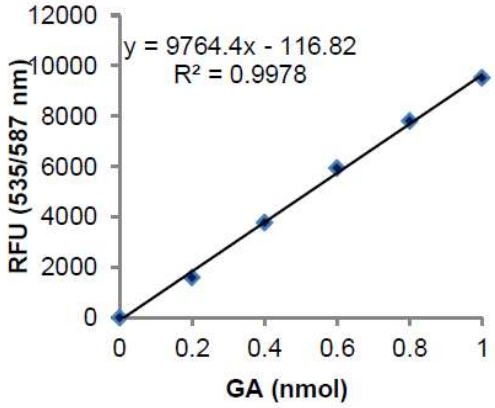


Figure 2.
Estimated GA concentrations in human serum samples. Spiked experiments show 90 ± 10% recovery. Assays were performed according to the kit protocol.

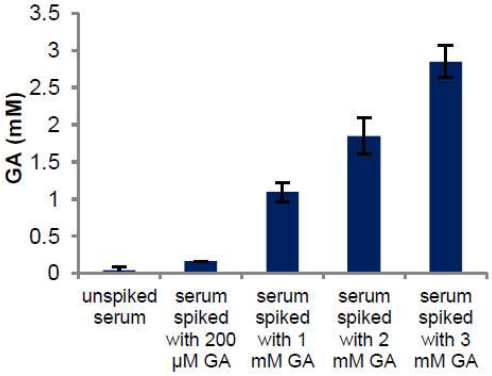
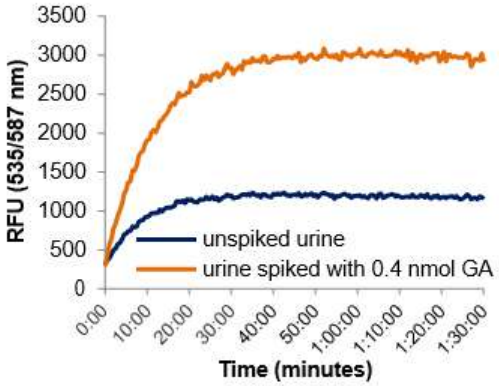


Figure 3.
Kinetic data for Urine sample, unspiked, and spiked with 0.4 nmol GA. GA was calculated to be 130 µM.



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