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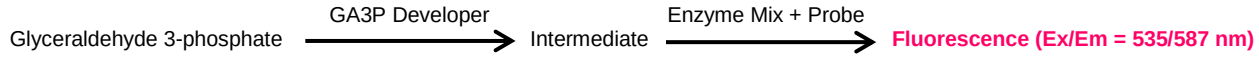
Glyceraldehyde 3-Phosphate Assay Kit (Fluorometric)

09/19

(Catalog # K2018-100; 100 assays; Store at - 20ºC)

I. Introduction:

Glyceraldehyde 3-phosphate (GA3P), also known as triose phosphate or 3-phosphoglyceraldehyde is an important metabolite that occurs as an intermediate central to several biochemical pathways such as glycolysis and gluconeogenesis. It is formed during glycolysis by the breakdown of fructose-1,6-biphosphate by the enzyme fructose biphosphate aldolase. It also occurs as a byproduct in tryptophan biosynthesis pathway or as a reactant in thiamine biosynthesis pathway. In plants, GA3P is the product of the Calvin Cycle and is the starting molecule for the synthesis of other carbohydrates. BioVision's Glyceraldehyde 3-Phosphate assay kit is a robust, one step plate based assay for the measurement of GA3P in biological samples. The GA3P developer oxidizes GA3P with the release of NADH, which is used by the GA3P enzyme mix to convert the non-fluorescent GA3P probe to a fluorescent product measured at Ex/Em = 535/587 nm.



II. Applications:

Quantification of Glyceraldehyde 3-phosphate

III. Sample Type:

- Tissue lysate (e.g. Liver tissue)
- Cell lysate

IV. Kit Contents:

Components	K2018-100	Cap Code	Part Number
GA3P Assay Buffer	25 ml	WM	K2018-100-1
GA3P Developer	1 vial	Green	K2018-100-2
GA3P Enzyme Mix	1 vial	Red	K2018-100-3
GA3P Probe	0.4 ml	Blue	K2018-100-4
GA3P Standard	1 vial	Yellow	K2018-100-5

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- 10 kDa spin columns (BV 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.

- **GA3P Assay Buffer:** Warm to room temperature (RT) before use.
- **GA3P Developer & GA3P Enzyme Mix:** Reconstitute each vial in 220 µl GA3P Assay Buffer. Aliquot and store at -20ºC in the dark. Thaw on ice before use.
- **GA3P Standard (20 mM):** Reconstitute in 1.5 ml distilled water to obtain a 20 mM GA3P Standard solution. Aliquot and store at -20°C. Thaw at RT before use.
- **GA3P Probe:** Thaw at RT, protect from light.

VII. GA3P Assay Protocol:

1. Sample Preparation:

a. Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl GA3P Assay Buffer to perform lysis. Keep on ice for 10 min followed by centrifugation at 10,000 x *g* and 4ºC for 15 min. Collect the supernatant (lysate) and estimate the protein concentration using any preferred method. We recommend using BCA protein assay kit (BV Cat# K813). Protein concentration should range between 0.05-1 µg/µl. Dilute the lysate if needed using GA3P Assay Buffer.

b. Various enzymes present in the sample can lead to a significant background, filter the samples using a 10 kDa spin columns (BV Cat# 1997) and use the ultrafiltrate for analysis.

c. For each Test Sample, add the same volume (2-15 µl) of Sample into three parallel wells in a white, flat bottom 96-well plate labeled as Sample Background Control, Unspiked Sample and Spiked Sample (containing Sample spiked with 200 pmol of GA3P Standard i.e. 8 µl of 25 µM G3P Standard solution). Adjust the volume to 50 µl/well with GA3P Assay Buffer.

d. For Assay Blank, add 50 µl GA3P Assay Buffer to a well(s).

Note: We recommend using the Samples for activity analysis immediately. Otherwise, store the Sample(s) at -80ºC for 3-4 days.

2. Internal Spike Preparation: Prepare a 25 µM GA3P Standard solution by diluting the 20 mM GA3P Standard stock solution at 1:800 dilution in water. Add 8 µl of the 25 µM GA3P Standard solution (200 pmoles GA3P Standard) to the Spiked Sample wells. Adjust the volume of all wells to 50 µl/well with GA3P Assay Buffer.

3. Reaction Mix Preparation: Mix enough Reaction Mix (used for both Unspiked Sample and Spiked Sample wells) and Background Mix (used 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.

	Reaction Mix	Background Mix
GA3P Assay Buffer	44 µl	46 µl
GA3P Developer	2 µl	-
GA3P Enzyme Mix	2 µl	2 µl
GA3P Probe	2 µl	2 µl

Mix well. Add 50 µl of the Reaction Mix to all Assay Blank, Unspiked Sample and Spiked Sample wells and 50 µl of the Background Mix to Sample Background Control wells.

Notes:

a) Have the microplate reader ready at Ex/Em = 535/587 nm in a kinetic mode at 37°C set to record fluorescence every 30 sec.

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

4. Measurement: Immediately start recording fluorescence in kinetic mode at 30 sec intervals for 60-90 min at 37°C.

5. Calculation: For both Unspiked and Spiked Sample wells, subtract the Sample Background Control (SBC) readings from Unspiked Sample (S) and Spiked Sample (SS) readings respectively. If "Assay Blank" readings are higher than SBC readings, then subtract those instead. Plot the data on MS Excel with time on the x-axis and RFU values on the y-axis respectively. Use the "Forecast" function to interpolate the data from 1 hr onwards to obtain the value of "y" at "x=0" for both S and SS wells, as shown in Fig **(b)**. Calculate the amount of GA3P in the Unspiked Sample wells using the following formula and using values obtained after interpolation.

$$\textbf{Amount of GA3P in Sample wells } (\boldsymbol{B}) = \frac{GA3P\ (Unspiked)}{GA3P\ (Spiked) - GA3P\ (Unspiked)}\ x\ 200\ pmol$$

Note: For Samples in which the calculated amount of GA3P is higher than 400 pmol, the Sample should be diluted further and tested again.

Calculate the GA3P concentration using the following**:**

$$\textbf{Sample GA3P Concentration} = \frac{B}{V} \times D = \textbf{pmol/µl = µM}$$

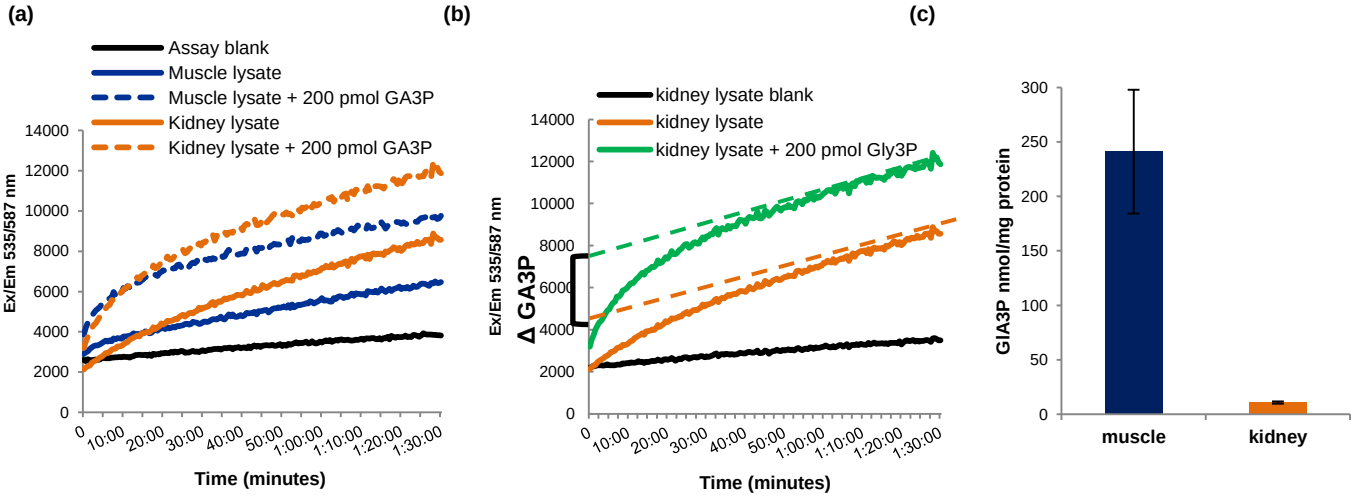
Where: ***B*** is the amount of GA3P calculated from the Standard addition formula above (in pmol)

V is the volume of Sample added to the well (in µl)

D is the sample dilution factor (if applicable, *D*= 1 for undiluted samples)

GA3P molecular weight: 170 g/mol

GA3P concentrations can also be expressed as nmol GA3P per mg protein.



Figures: (a) Kinetics of enzymatic reaction to measure GA3P in rat muscle (608 ng) and mouse kidney (8.5 µg) lysate with and without GA3P Standard spikes. **(b)** Interpolation of kinetic data for mouse kidney lysate. **Δ GA3P = (GA3P spiked – GA3P unspiked)**. **(c)** GA3P in rat muscle and mouse kidney lysates. Experiments were conducted according to kit protocol.

VIII. Related Products:

PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glycerol-3-Phosphate (G3P) Colorimetric Assay Kit (K641)
GAPDH Activity Assay Kit (K680)

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