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ab273344 Glyceraldehyde 3-Phosphate Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273344
(use https://www.abcam.cn/ab273344 for china, or
https://www.abcam.co.jp/ab273344 for Japan)

For the determination of Glyceraldehyde 3-Phosphate in adherent/suspension cells and tissue.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Glyceraldehyde 3-Phosphate Assay Kit (Fluorometric) (ab273344) is a robust assay for the measurement of Glyceraldehyde 3-Phosphate (GA3P) in biological samples. The Developer Mix K oxidizes GA3P with the release of NADH, which is used by the Developer Mix E to convert the non-fluorescent PicoProbe I to a fluorescent product measured at Ex/Em = 535/587 nm.

2. Protocol Summary

Prepare all reagents, samples and internal spike.

Add all samples/spikes to the appropriate wells.

Prepare and add Reaction Mix and Background Mix to the appropriate wells.

Measure fluorescence immediately in kinetic mode at 30 second intervals for 60-90 mins at 37°C.

Determine Glyceraldehyde 3-Phosphate amount using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
GA3P Assay Buffer	25 mL	-20°C
Developer Mix K	1 vial	-20°C
Developer Mix E	1 vial	-20°C
PicoProbe I	0.4 mL	-20°C
Glyceraldehyde-3-Phosphate	200 µL	-20°C

PLEASE NOTE: Developer Mix K was previously labelled as Developer Mix V and GA3P Developer Lyophilized, and Developer Mix E as Development Enzyme Mix VI and GA3P Enzyme Mix Lyophilized. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm (temperature-controlled)
- 96-well white plate with flat bottom
- dH_2O
- 10kDa spin columns

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **GA3P Assay Buffer:**

Ready to use as supplied. Bring to room temperature before use.

9.2 **Developer Mix K:**

Reconstitute vial contents with 220 µl GA3P Assay Buffer. Aliquot and store at -20°C in the dark. Thaw on ice before use.

9.3 **Developer Mix E:**

Reconstitute vial contents with 220 µl GA3P Assay Buffer. Aliquot and store at -20°C in the dark. Thaw on ice before use.

9.4 **PicoProbe I:**

Ready to use as supplied. Thaw at RT protect from light.

9.5 **Glyceraldehyde-3-Phosphate:**

Reconstitute in 1.3 ml distilled water to obtain a 20 mM GA3P Standard solution. Aliquot and store at -20°C. Thaw at RT before use.

10. Sample Preparation

Tissue/cell lysate preparation:

10.1 Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl GA3P Assay Buffer to perform lysis.
10.2 Keep on ice for 10 mins followed by centrifugation at 10,000 *x g* and 4°C for 15 mins.
10.3 Collect the supernatant (lysate) and estimate the protein concentration using any preferred method. We recommend using a BCA protein assay kit.
10.4 Protein concentration should range between 0.05-1 µg/µl. Dilute the lysate if needed using GA3P Assay Buffer.
10.5 Various enzymes present in the sample can lead to a significant background, filter the samples using a 10 kDa spin columns and use the ultrafiltrate for analysis.
10.6 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, Un-spiked Sample and Spiked Sample (containing Sample spiked with 200 pmol of GA3P Standard i.e. 8 µl of 25 µM G3P Standard solution).
10.7 Adjust the volume to 50 µl/well with GA3P Assay Buffer. For Assay Blank, add 50 µl GA3P Assay Buffer to a well(s).

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

11. Assay Procedure

- 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.
- Prepare Reaction Mix immediately before adding to the wells.

11.1 **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.

11.2 **Reaction Mix:** Prepare enough Reaction Mix (used for both Un-spiked 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 (µl)	Background mix (µl)
GA3P Assay Buffer	44	46
Developer Mix K	2	---
Developer Mix E	2	2
PicoProbe I	2	2

11.3 Add 50 µl of the Reaction Mix to all Assay Blank, Un-spiked Sample and Spiked Sample wells.

11.4 Add 50 µl of the Background Mix to Sample Background Control wells.

11.5 Immediately start recording fluorescence in kinetic mode at 30 second intervals for 60-90 mins at 37°C.

12. Calculations

12.1 For both Un-spiked and Spiked Sample wells, subtract the Sample Background Control (SBC) readings from Un-spiked Sample (S) and Spiked Sample (SS) readings respectively.

12.2 If 'Assay Blank' readings are higher than SBC readings, then subtract those instead.

12.3 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 hour onwards to obtain the value of 'y' at 'x=0' for both S and SS wells, as shown in Fig 2.

12.4 Calculate the amount of GA3P in the Un-spiked Sample wells using the following formula and using values obtained after interpolation:

$$GA3P\ amount = \frac{GA3P\ unspiked}{(GA3P\ spiked - GA3P\ unspiked)}\ X\ 200\ pmol$$

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

12.5 Calculate the GA3P concentration using the following:

$$Sample\ GA3P\ conc = \frac{B}{V}\ X\ D = pmol/\mu l$$

B = is the amount of GA3P calculated from the Standard addition formula above (pmol)
V = is the volume of Sample added to the well (µl)
D = is the sample dilution factor (if applicable, D=1 for undiluted samples)

pmol/µl = µM

GA3P molecular weight: 170 g/mol
GA3P concentrations can also be expressed as nmol GA3P per mg protein.

13. Typical Data

Typical data – data provided **for demonstration purposes only.**

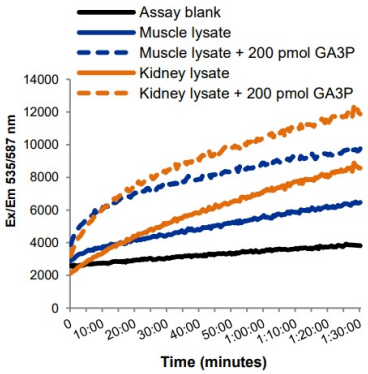

Figure 1. Kinetics of enzymatic reaction to measure GA3P in rat muscle (608 ng) and mouse kidney (8.5 µg) lysate with and without GA3P Standard spikes.

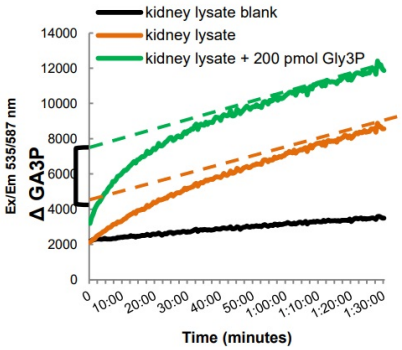

Figure 2. Interpolation of kinetic data for mouse kidney lysate. Δ GA3P = (GA3P spiked – GA3P unspiked).

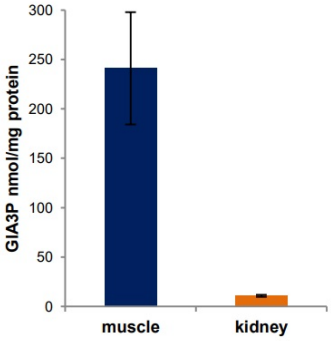

Figure 3. GA3P in rat muscle and mouse kidney lysates.

14. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)