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ab273271 Glycogen Phosphorylase Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273271
(use https://www.abcam.cn/ab273271 for china, or
https://www.abcam.co.jp/ab273271 for Japan)

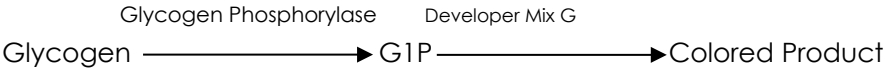
For the determination of Glycogen Phosphorylase activity in animal tissues and cultured cells.

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

Table of Contents

1. Overview

Glycogen Phosphorylase Assay Kit (Colorimetric) (ab273271) is based on the detection of G1P by a set of enzymatic reactions to generate a colored product with a strong absorbance at 450 nm. The OD 450 nm signal is directly proportional to the glycogen phosphorylase activity. The Glycogen Phosphorylase Colorimetric Assay Kit is rapid, sensitive and convenient tool for detecting glycogen phosphorylase activity. The kit can detect as low as 10 mU in a variety of sample types.



2. Protocol Summary

Prepare lysates as directed and measure protein concentration

Prepare all reagents as directed

Add Positive Control, Samples and Background Control to appropriate wells

Add Reaction Mix (to Samples, Standards and Positive Control) and Background Reaction Mix (Background Control) to appropriate wells

Measure absorbance at 450 nm in kinetic mode for 60 minutes at 30°C.

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. The kit components are stable for one year when stored as recommended.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied 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)
Assay Buffer 37	20 mL	-20°C
Enzyme Mix XVII	1 vial	-20°C
Developer Mix G	1 vial	-20°C
Developer Solution III	1 vial	-20°C
G1P Standard	1 vial	-20°C
Glycogen Substrate	1 vial	-20°C
Glycogen Phosphorylase	1 vial	-20°C

PLEASE NOTE: Assay Buffer 37 was previously labelled as Assay Buffer XXXVII and Assay Buffer, and Glycogen Substrate as Glycogen (Lyophilized), and Developer Mix G as Development Enzyme Mix IX and Developer (Lyophilized), and Enzyme Mix XVII as Enzyme Mix (Lyophilized), and Developer Solution III as Substrate 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:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- 50% Glycerol
- PBS

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 Assay Buffer 37

Ready to use as supplied. Warm bottle to room temperature before use. Store at -20°C.

9.2 Enzyme Mix XVII:

Reconstitute with 220 µL of Assay Buffer 37. Pipette up and down to dissolve completely. Keep on ice while in use. Store at -20°C and use within 2 months.

9.3 Developer Mix G:

Reconstitute with 220 µL of Assay Buffer 37. Pipette up and down to dissolve completely. Keep on ice while in use. Store at -20°C and use within 2 months.

9.4 Developer Solution III:

Reconstitute with 220 µL of Assay Buffer 37. Pipette up and down to dissolve completely. Keep on ice while in use. Store at -20°C and use within 2 months.

9.5 G1P Standard:

Reconstitute with 100 µL of water to generate 100 mM G1P. Store at -20°C and use within 2 months.

9.6 Glycogen Substrate:

Reconstitute with 1.2 mL of water. Pipette up and down to dissolve. Keep cold while in use. Store at -20°C and use within 2 months.

9.7 Glycogen Phosphorylase:

Reconstitute with 50 µL of 50% glycerol (not included). Keep cold while in use. Aliquot and store at -20°C. Avoid repeated freeze thaw cycles.

10. Sample Preparation

Animal tissue and cells:

10.1 Homogenize tissue (50 mg) or cells (10^6 cells) with 200 µL of ice cold Assay Buffer 37.

10.2 After homogenization, keep the lysates on ice for 15 min.

10.3 Centrifuge at 10,000 x g and 4°C for 15 min. Transfer the clear sample supernatant to a new tube.

10.4 For supernatants prepared from tissues, use PBS to further dilute the supernatant.

10.5 For each tested sample prepare two parallel wells: Sample (S) and Background Control (BC (see Section 12).

11. Standard Curve

11.1 Mix 10 µl of 100 mM G1P with 990 µL of water to prepare 1 mM G1P Standard. Keep on ice while in use.

11.2 Add 0, 2, 4, 6, 8 and 10 µL of the 1 mM G1P Standard into the desired wells to generate 0, 2, 4, 6, 8 and 10 nmole of G1P per well, respectively.

11.3 Adjust the volume to 50 µL with Assay Buffer 37.

Standard #	1 mM G1P Standard (µL)	Assay Buffer 37 (µL)	G1P Standard (nmol/well)
1	10	40	10
2	8	42	8
3	6	44	6
4	4	46	4
5	2	48	2
6	0	50	0

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of the appropriate Reaction Mix containing:

Reaction mixes:

Prepare enough reagents for the number of assays to be performed.

Component	Reaction Mix	Background Reaction Mix
Assay Buffer 37	34 µL	44 µL
Glycogen Substrate	10 µL	-
Enzyme Mix XVII	2 µL	2 µL
Developer Mix G	2 µL	2 µL
Developer Solution III	2 µL	2 µL

12.1.2 **Samples:** For each tested sample prepare two parallel wells: Sample (S) and Background Control (BC) wells by adding 2 µL of the supernatant (see Section 10) to the desired wells in a 96-well clear flat-bottom plate. Adjust the volume to 50 µL with Assay Buffer 37.

12.1.3 **Positive Control:** For Positive Control (PC) well, add 2 µL of the glycogen phosphorylase enzyme into designated wells in the plate. Adjust the volume to 50 µL with Assay Buffer 37.

Δ Note: For Unknown Samples, we suggest doing a pilot experiment and testing several dilutions to ensure the readings are within the Standard Curve range.

12.1.4 To start the reaction, add 50 µL of Reaction Mix to each well containing Standards, Samples (S) or Positive Control (PC).

12.1.5 To all Sample Background Controls (BC) add 50 µL of Background Mix to the desired Sample wells.

12.1.6 Measure the OD at 450 nm in kinetic mode at 30°C for 60 min. After the reaction completes, the OD 450 nm signal may

start to decrease. Therefore use the maximum OD 450 nm for calculation

13. Calculations

13.1 Subtract 0 pmol Standard from all Standard readings.
13.2 Plot the G1P Standard Curve.
13.3 Select two time points within the linear portion of the curve t_1 and t_2.
13.4 Subtract the Sample Background OD reading from Sample reading for these two time points.
13.5 Calculate the glycogen phosphorylase activity of the Sample: ΔOD 450 nm = $OD_2 - OD_1$ at time points t_2 and t_1.
13.6 Apply the ΔOD 450 nm to the G1P standard curve to get A nmol of G1P generated during the reaction time ($\Delta t = t_2 - t_1$).

$$Specific\ activity = \frac{A\ X\ D}{(\Delta t\ X\ M)}\ \ (mU/mg)$$

A = G1P from Sample calculated using the Std Curve equation (nmol)
Δt = Reaction time
D = Dilution Factor
M = Sample used (mg)

Unit definition:

One unit is 1 µmole of G1P generated per min at pH 7.0 and 30°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

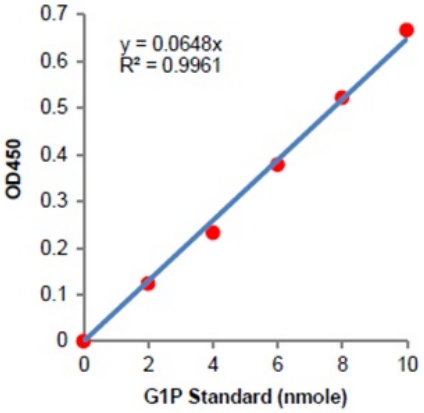


Figure 1. G1P Standard Curve.

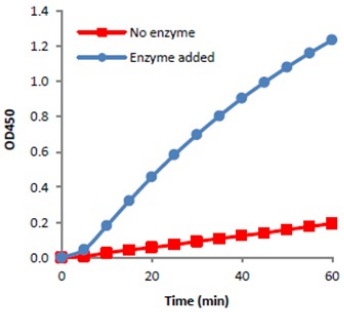


Figure 2. Reaction curves of Glycogen Phosphorylase versus no enzyme control.

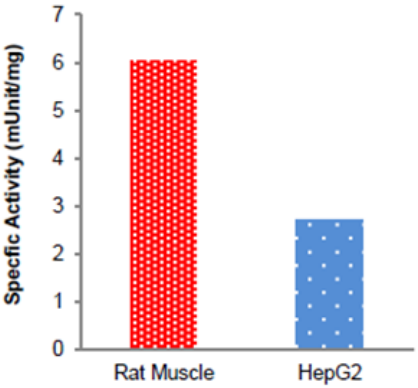


Figure 3. Specific activity of Glycogen Phosphorylase in different sample types.

15. FAQ / Troubleshooting

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

16. 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)