Technical Bulletin

Glycogen Phosphorylase Colorimetric Assay Kit

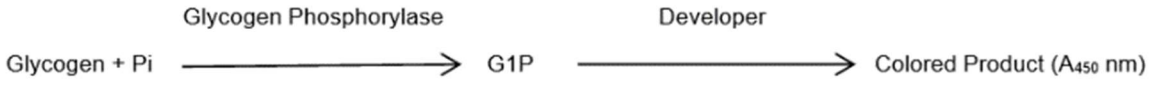
Catalog Number MAK417

Product Description

Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis using glycogen and inorganic phosphate to produce glucose-1-phosphate (G1P). In mammals, glycogen phosphorylase is abundant in muscle, liver, and brain tissues. There are two forms of glycogen phosphorylase, namely glycogen phosphorylase a and b forms. Glycogen phosphorylase a is the highly active form whereas glycogen phosphorylase b has only limited activity. Glycogen phosphorylase is a clinically significant enzyme as its mutations are associated with different glycogen storage diseases in muscle and liver. In addition, this enzyme has been suggested as a biomarker for gastric cancer and its inhibition has been tested in treating type 2 diabetes.

The Glycogen Phosphorylase Colorimetric Activity Assay Kit 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 450 nm signal (A_{450}) is directly proportional to the glycogen phosphorylase activity. The kit is a 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.

The kit is suitable for the measurement of glycogen phosphorylase activity in cell culture and animal tissues (heart, liver, kidney, muscle, etc.) as well as for the analysis of glycogen phosphorylase kinetics, inhibition, or activation.



Components

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

- Assay Buffer — 20 mL
  Catalog Number MAK417A
- Enzyme Mix — 1 vial
  Catalog Number MAK417B
- Developer — 1 vial
  Catalog Number MAK417C
- Substrate Mix — 1 vial
  Catalog Number MAK417D
- G1P Standard — 1 vial
  Catalog Number MAK417E
- Glycogen — 1 vial
  Catalog Number MAK417F
- Glycogen Phosphorylase — 1 vial
  Catalog Number MAK417G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Glycerol (Catalog Number G7757 or equivalent)
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)

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 kit at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Assay Buffer: Ready to use as supplied. Warm bottle to room temperature prior to use. Store at 2-8 °C.

Enzyme Mix, Developer and Substrate Mix: Reconstitute Enzyme Mix, Developer and Substrate Mix vials with 220 µL of Assay Buffer separately. Pipette up and down to dissolve completely. Keep on ice while in use. Store at -20 °C and use within two months once reconstituted.

G1P Standard: Reconstitute vial with 100 µL of purified water to generate 100 mM G1P. Store at -20 °C and use within two months once reconstituted.

Glycogen: Reconstitute vial with 1.2 mL of purified water. Pipette up and down to dissolve. Keep cold while in use. Store at -20 °C and use within two months once reconstituted.

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.

Procedure

All samples, controls, and standards should be run in duplicate.

Sample Preparation

1. Homogenize tissue (50 mg) or cells (1×10^6 cells) with 200 µL of ice-cold Assay Buffer.
2. After homogenization, keep the lysates on ice for 15 minutes.
3. Centrifuge at 10,000 × *g*, 4 °C for 15 minutes.
4. Transfer the clear sample supernatant to a new tube.
5. For supernatants prepared from tissues, use PBS (not included) to further dilute the supernatant. For unknown samples, perform a pilot experiment by testing several dilutions to ensure the readings are within the Standard Curve range.
6. For each tested sample prepare two parallel wells: Sample (S) and Background Control (BC) wells by adding 2 µL of the supernatant to the desired wells in a 96-well clear flat-bottom plate.
7. Adjust the total volume of each well to 50 µL with Assay Buffer.

Positive Control (PC)
Add 2 µL of Glycogen Phosphorylase enzyme into designated wells in the plate. Adjust the total volume to 50 µL with Assay Buffer.

Standard Curve Preparation

Prepare a 1 mM G1P Solution by adding 10 µL of the 100 mM G1P Standard to 990 µL of purified water. Keep on ice while in use. Prepare G1P standards according to Table 1, mix well.

Table 1.
Preparation of G1P Standards

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

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed. For each well containing Standard, Sample (S) and Positive Control (PC), prepare 50 µL of Reaction Mix. For each well containing Background Control (BC), prepare 50 µL of Background Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Reaction Mix
Assay Buffer	34 µL	44 µL
Glycogen	10 µL	-
Enzyme Mix	2 µL	2 µL
Developer	2 µL	2 µL
Substrate Mix	2 µL	2 µL

2. To start the reaction, add 50 µL of Reaction Mix to each well containing Standards, Samples (S) or Positive Control (PC). To all Sample Background Controls (BC) wells, add 50 µL of Background Reaction Mix.

Measurement
Measure the absorbance at 450 nm (A_{450}) in kinetic mode at 30 °C for 60 minutes. After the reaction completes, the A_{450} signal may start to decrease. Therefore, use the maximum A_{450} for calculation.

Results

1. Subtract 0 Standard Reading from all Standard Readings.
2. Plot the G1P Standard Curve.
3. Select two time points within the linear portion of the curve T_1 and T_2.
4. Subtract the Sample Background Control (BC) A_{450} reading from Sample (S) reading for these two time points.
5. Calculate the glycogen phosphorylase activity of the Sample:

$$\Delta A_{450} = A_{450\ T1} - A_{450\ T2}$$
at time points T_1 and T_2

6. Apply the ΔA_{450} to the G1P standard curve to get N nmol of G1P generated during the reaction time ($\Delta T = T_2 - T_1$).

$$\text{Specific Activity (mU/mg)} = \frac{(N \times D)}{(\Delta T \times M)}$$

where:

N = G1P from Standard Curve (nmol)
D = Dilution factor
ΔT = Reaction time (minutes)
M = Sample used (mg)

Unit Definition: One unit of glycogen phosphorylase generates 1 µmole of G1P per minute at pH 7.0 and 30 °C.

Figure 1.
Typical G1P Standard Curve.

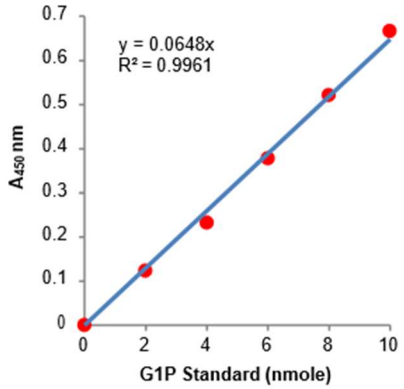


Figure 2.
Reaction curves of glycogen phosphorylase vs. control with no added enzyme.

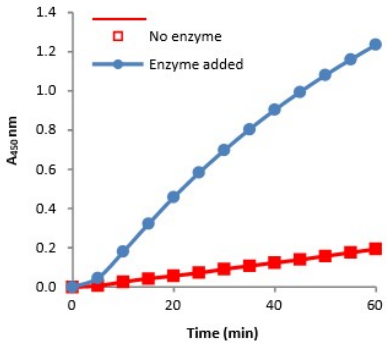
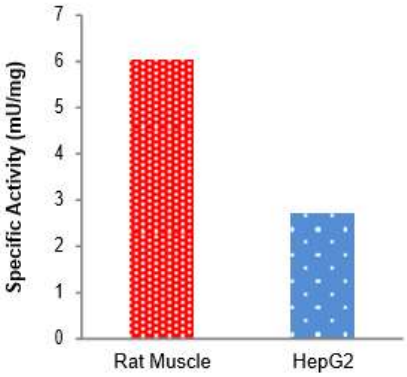


Figure 3.
Specific activity ofglycogen phosphorylase determined in different samples using the kit protocol.



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