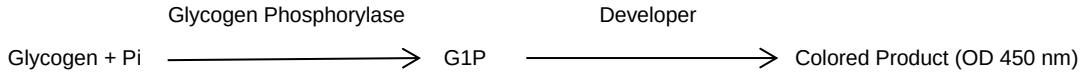
Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Glycogen Phosphorylase Colorimetric Assay Kit

(Catalog # K179-100; 100 assays, Store kit at -20°C)

I. Introduction:

Glycogen phosphorylase (EC 2.4.1.1) 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 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. BioVision's Glycogen Phosphorylase 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 OD 450 nm signal is directly proportional to the glycogen phosphorylase activity. BioVision's 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.



II. Application:

- Measurement of glycogen phosphorylase activity in various tissues or cells
- Analysis of glycogen phosphorylase kinetics, inhibition or activation

III. Sample Type:

- Cell culture and animal tissues (heart, liver, kidney, muscle, etc.).

IV. Kit Contents:

Components	K179-100	Cap Code	Part Number
Assay Buffer	20 ml	WM	K179-100-1
Enzyme Mix	1 vial	Purple	K179-100-2
Developer	1 vial	Green	K179-100-3
Substrate Mix	1 vial	Red	K179-100-4
G1P Standard	1 vial	Yellow	K179-100-5
Glycogen	1 vial	Orange	K179-100-6
Glycogen Phosphorylase	1 vial	Blue	K179-100-7

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **Assay Buffer:** Ready to use as supplied. Warm bottle to room temperature before use. Store at 4°C.
- **Enzyme Mix, Developer and Substrate Mix:** Reconstitute Enzyme Mix, Developer and Substrate Mix 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 2 months.
- **G1P Standard:** Reconstitute with 100 µl of water to generate 100 mM G1P. Store at -20°C and use within 2 months.
- **Glycogen:** 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.
- **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.

VII. Glycogen Phosphorylase Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (50 mg) or cells (10^6 cells) with 200 µl of ice cold Assay Buffer. After homogenization, keep the lysates on ice for 15 min. Centrifuge at 10,000 x *g* and 4°C for 15 min. Transfer the clear sample supernatant to a new tube. For supernatants prepared from tissues, use PBS to further dilute the supernatant. 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. Adjust the volume to 50 µl with Assay Buffer. 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.
   Notes: For Unknown Samples, we suggest doing a pilot experiment & testing several dilutions to ensure the readings are within the Standard Curve range.
2. **Standard Curve Preparation:** Mix 10 µl of 100 mM G1P with 990 µl of water to prepare 1 mM G1P Standard. Keep on ice while in use. 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. Adjust the volume to 50 µl with Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare 50 µl of Reaction Mix and 50 µl of Background Mix as indicated in the table below:

	Reaction Mix	Background 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

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**) add 50 µl of Background Mix to the desired Sample wells.

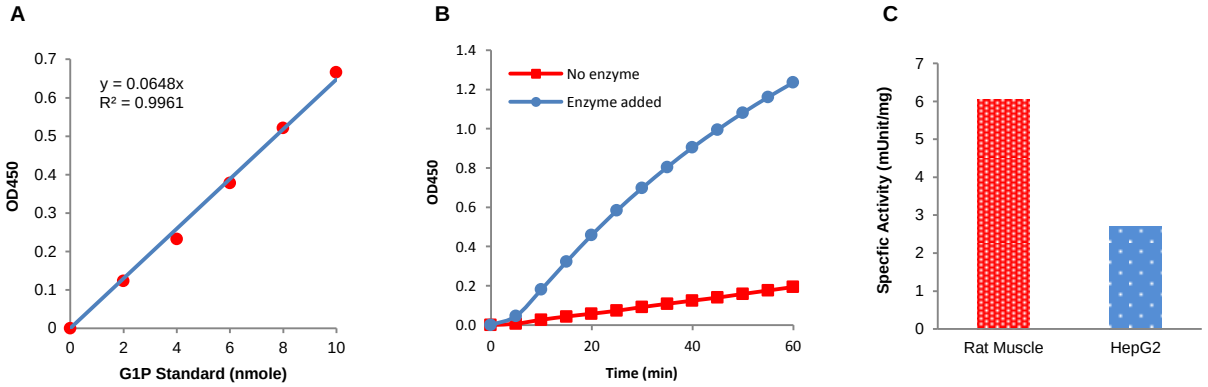
4. **Measurement:** Measure the OD at 450 nm in kinetic mode at 30°C for 60 min. After the reaction completes, the OD 450 nm signal may start to decrease. Therefore use the maximum OD 450 nm for calculation.

5. **Calculation:** Subtract 0 Standard Reading from all Standard Readings. Plot the G1P Standard Curve. Select two time points within the linear portion of the curve t_1 and t_2. Subtract the Sample Background OD reading from Sample reading for these two time points. Calculate the glycogen phosphorylase activity of the Sample: $\Delta OD\ 450\ nm = OD_1 - OD_2$ at time points t_1 and t_2. 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 = A x D / (Δt x M) = (mU/mg)

Where: A = G1P from Standard Curve (nmol)
Δt = Reaction time (min)
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.



Figures A. G1P Standard Curve. **B.** Reaction curves of glycogen phosphorylase vs. no enzyme control in the assay. **C**. Specific activity of glycogen phosphorylase determined in different samples using the kit protocol.

VIII. RELATED PRODUCTS:

Ethanolamine Kinase 2, human recombinant (P1158)
Phospholipid Assay Kit (Colorimetric/Fluorometric) (K351)
Phospholipase D (PLD) Activity Colorimetric Assay Kit (K725)
Pyruvate Colorimetric/Fluorometric Assay Kit (K609)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
Choline Kinase B, Human Recombinant (P1220)
Phospholipase A2 Activity Assay Kit (Fluorometric) (K400)
Malate Colorimetric Assay Kit (K637)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)

FOR RESEARCH USE ONLY! Not to be used on humans.