ab308153 – Glycogen Phosphorylase Activity Assay Kit (Fluorometric)

For the measurement of glycogen phosphorylase activity in various samples.

For overview, typical data and additional information please visit: www.abcam.com/ab308153
(use www.abcam.cn/ab308153 for China, or www.abcam.co.jp/ab308153 for Japan)

Storage and Stability: All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
Reconstituted components are stable for 2 months.
Aliquot components in working volumes before storing at the recommended temperature.
Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 37	25 mL	-20 °C or +4 °C	+4 °C
Glycogen	1 vial	-20 °C	-20 °C
PicoProbe II	0.2 mL	-20 °C	-20 °C
Enzyme Mix XVII	1 vial	-20 °C	-20 °C
Developer Mix G	1vial	-20 °C	-20 °C
Developer Solution X	1vial	-20 °C	-20 °C
Glycogen Phosphorylase	1vial	-20 °C	-20 °C
G1P Standard	1vial	-20 °C	-20 °C

PLEASE NOTE: Developer Mix G was previously labelled as Development Enzyme Mix IX, and Assay Buffer 37 as Assay Buffer XXXVII. The composition has not changed.

Materials Required, Not Supplied

- Microplate reader capable of measuring fluorescence at Ex/Em = 538/587 nm (fluorometric)
- White 96 well plate with flat bottom
- Dounce homogenizer (if using tissue)
- Reagent grade glycerol

Reagent Preparation:
Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer 37: Ready to use. Equilibrate to room temperature (RT).

Glycogen: Reconstitute the vial with 1.2 ml of water. Divide into aliquots and store at -20 °C. Prior to use, thaw aliquots at 37 °C and pipette up and down to dissolve.

PicoProbe II: Provided as a stock solution in DMSO. Divide into aliquots and store at -20 °C, protected from light. Prior to use, warm solution to RT and vortex thoroughly.

Enzyme Mix XVII, Developer Mix G, Developer Solution X: Reconstitute each of the vials with 220 µl Assay Buffer 37. Divide each into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

Glycogen Phosphorylase: Reconstitute the vial with 100 µl of 50% dH2O / 50% glycerol (not included) to prepare Glycogen Phosphorylase stock. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

G1P Standard: Reconstitute the vial with 100 µl dH_2O to yield a 100 mM G1P Standard stock solution. Divide into aliquots and store at -20 °C. Stable for at least 3 freeze/thaw cycles.

Sample Preparation

ΔNOTE: We recommend using different dilutions and/or volumes of sample to ensure readings are within the standard curve range.
ΔNOTE: To quantify Glycogen Phosphorylase specific activity in terms of protein content tissue sample lysates, we recommend measuring sample protein concentration using protein quantitation Kit (ab102535) or any equivalent protein assay.

Tissue Samples:

1. Homogenize tissue (50 mg wet tissue) with 250 µl of ice cold Assay Buffer 37 using a Dounce homogenizer or a probe sonicator.
2. After homogenization, transfer tissue homogenates to a microfuge tube, vortex thoroughly and place on ice for 5 min.
3. Centrifuge at 10,000 x g and 4 °C for 15 min.
4. Transfer the clarified sample supernatant to a new microfuge tube.

ΔNOTE Tissue homogenates may be stored at -20 °C in aliquots for future experiments. Harvest the necessary amount of tissue for each assay (~100 mg of tissue).

5. For each sample, prepare two parallel wells, with one well serving as a Sample Background Control. Add 2-40 µl of the clarified supernatant to the desired wells in a white, flat-bottomed 96-well plate.
6. Adjust the volume of each well to 50 µl with Assay Buffer 37.

Positive Control:

7. Dilute the Glycogen Phosphorylase by adding 5 µl of the reconstituted Glycogen Phosphorylase stock to 495 µl Assay Buffer 37. Then add 5-10 µl of diluted Glycogen Phosphorylase solution into the desired well(s).
8. Adjust the volume to 50 µl with Assay Buffer 37.

ΔNOTE: Once diluted, the Glycogen Phosphorylase should be used within 2 hours. Do not freeze the diluted Glycogen Phosphorylase.

Standard Preparation
Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

1. Prepare a 1 mM G1P Standard working solution by adding 10 µl of the 100 mM G1P Standard stock to 990 µl of dH_2O.

2. Further dilute the 1 mM G1P Standard working solution to 50 µM G1P Standard solution by adding 10 µl to 190 µl of Assay Buffer 37. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM G1P Standard solution into a series of wells to generate 0, 100, 200, 300, 400 and 500 pmoles of Standard/well.
3. Adjust the volume of all G1P Standard wells to 50 µl with Assay Buffer 37.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL).

Standard #	50 µM G1P Standard (µL)	Assay Buffer 37 (µL)	Final volume standard in well (µL)	G1P in well (pmol)
1	0	125	50	0
2	5	120	50	100
3	10	115	50	200
4	15	110	50	300
5	20	105	50	400
6	25	100	50	500

Assay Procedure

Equilibrate all materials and prepared reagents to RT just prior to use and gently agitate.
Assay all standards, controls and samples in duplicate.
Endogenous compounds in the sample may interfere with the reaction so we recommend spiking samples with a known amount of standard (2 – 10 nmol).
If you suspect your samples contain glycerol, set up Sample Background Controls to correct for background noise which may be caused by interference of glycerol with lipase activity.

1. Preincubate the plate for 10 min at 37 °C to allow for temperature equilibration. During the preincubation, prepare the Reaction Mix and Sample Background Mix according to the table below. Prepare sufficient volume of each type of mix to add 50 µl to all assay wells of that type. Remember to account for the Standard Curve wells when calculating the amount of Reaction Mix to prepare.

Component	Reaction Mix (µL)	Sample Background Mix (µL)
Assay Buffer 37	33	43
Glycogen	10	-
PicoProbe II	1	1
Enzyme Mix XVII	2	2
Developer Mix G	2	2
Developer Solution X	2	2

2. Add 50 µl of the Reaction Mix to all Sample, Positive Control (if applicable) and Standard Curve wells.
3. Add 50 µl of the Sample Background Mix to all Sample Background Control well(s), yielding a final volume of 100 µl per well.
4. Measure the fluorescence at Ex/Em = 538/587 nm of all sample wells in kinetic mode for 60 min at 37 °C.

ΔNOTE: We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample. The G1P Standard Curve wells may be read in endpoint mode (Ex/Em = 538/587 nm).

Data analysis

1. Subtract the 0 pmol/well Standard reading from all of the Standard readings and plot the background-subtracted Standard values and calculate the slope of the G1P Standard Curve.
2. For all reaction wells, choose any two time points (t_1 and t_2) in the linear phase of the reaction progress curves and obtain the corresponding fluorescence values (RFU_1 and RFU_2). Determine the change in fluorescence over the time interval ($\Delta F = RFU_2 - RFU_1$).
3. Subtract the ΔF value of the Sample Background Control (ΔF_{BC}) from that of the corresponding test Sample (ΔF_S) well to obtain the specific change in fluorescence intensity generated by Glycogen Phosphorylase activity (denoted by F_C) for each sample:

$$\mathbf{F_C} = \Delta F_S - \Delta F_{BC}$$

ΔNOTE: Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

4. Apply the FC values to the G1P Standard Curve to get B pmol of substrate metabolized by Glycogen Phosphorylase during the reaction time:

Sample Glycogen Phosphorylase Activity = B/(ΔT×P) = pmol/(min*mg)

Where:

B is the amount of peptide substrate cleaved, calculated from the G1P Standard Curve (in pmoles)
ΔT is the linear phase reaction time $t_2 - t_1$ (in min)
P is the amount of sample added to the well (in mg of tissue/protein)

ΔNOTE: Remember to account for any dilution of the sample made during sample preparation when determining P.

Glycogen Phosphorylase Unit Definition: One unit of Glycogen Phosphorylase activity is the amount of enzyme that generates 1 µmol of G1P per min by hydrolysis of 1 µmol glycogen at 37 °C and pH 7.5.

Technical Support

For all technical or commercial enquiries please go to:
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