Version 3a, Last updated 18 August 2023

ab211104 Phosphoglucomutase Activity Assay Kit

For the rapid, sensitive and accurate measurement of Phosphoglucomutase (PGM) activity in cell and tissue lysates.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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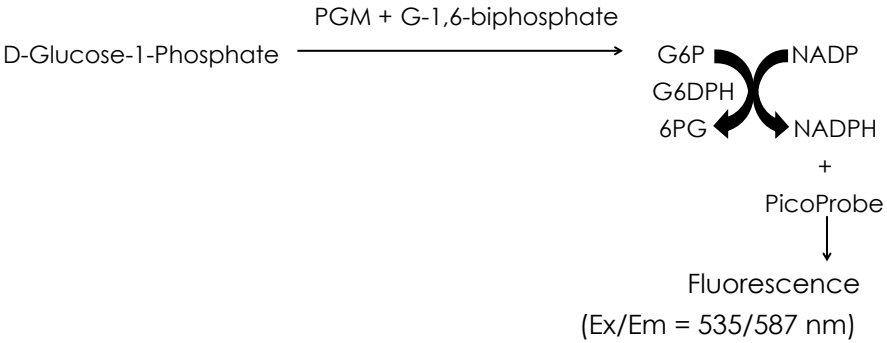
1. Overview

Phosphoglucomutase Activity Assay Kit (Fluorometric) (ab211104) provides a sensitive, simple and convenient method for measuring Phosphoglucomutase (PGM) activity in plasma, cell and tissue lysates. The assay is based on the ability of Phosphoglucomutase to convert glucose-1-phosphate to glucose-6-phosphate. Glucose-6-phosphate is then oxidized by Glucose-6-phosphate dehydrogenase (G6PDH) to form NADPH, which in turn reduces the probe to generate an intense fluorescence product that can detected at Ex/Em = 535/587 nm.

This assay can detect as low as 20 µU of Phosphoglucomutase activity.



Phosphoglucomutase (PGM, EC 5.4.2.2) facilitates the interconversion of glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P). Glycogen breakdown generates G1P, which is converted to G6P by PGM. G6P can then go into the glycolytic pathway to generate ATP, or to the pentose phosphate pathway to generate ribose and NADPH. On the other hand, when cells have extra energy, G6P is converted by PGM into G1P to generate glycogen.

In humans, two isoforms have been described, PGM I and PGM II. PGM deficiency leads to glucose storage disease.

2. Protocol Summary

Standard curve preparation

Sample preparation

Add reaction mix

Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 10 – 60 minutes at 25°C.

**For kinetic mode detection, incubation time given in this summary is for guidance only*

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. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

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.

Δ Note: Reconstituted components are stable for 2 months.

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)	Storage temperature (after prep)
Assay Buffer V/PGM Assay Buffer	25 mL	-20°C	4°C / -20°C
NADPH Standard/NADPH Standard (0.2 µmol)	1 vial	-20°C	-20°C
Developer IX/PGM Developer (30 U)	1 vial	-20°C	-20°C
Development Enzyme Mix IX/PGM Enzyme Mix (10 U)	1 vial	-20°C	-20°C
PGM Positive Control/PGM Positive Control (10 U)	1 vial	-20°C	-20°C
PGM Substrate/PGM Substrate (10 µmol)	1 vial	-20°C	-20°C
PicoProbe I/PicoProbe (in DMSO)	400 µL	-20°C	-20°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably white
- Dounce homogenizer (if using tissue)
- Optional: BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)
- Optional: Protease Inhibitor Cocktail (ab65621)

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 which generate 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 V/PGM Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 Developer IX/PGM Developer (lyophilized, 30 U):

Reconstitute in 220 µL Assay Buffer. Pipette up and down to dissolve completely. Aliquot developer so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.3 Development Enzyme Mix IX/PGM Enzyme Mix (lyophilized, 10 U):

Reconstitute in 220 µL Assay Buffer. Pipette up and down to dissolve completely. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Use with 2 months.

9.4 PGM Positive Control/PGM Positive Control (lyophilized, 10 U):

Reconstitute in 100 µL Assay Buffer. Mix thoroughly by pipetting up and down. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

Immediately before performing the assay, dilute PGM positive control 1:1000 in Assay Buffer. Do not store diluted positive control.

9.5 PGM Substrate/PGM Substrate (lyophilized, 10 µmol):

Reconstitute in 220 µL Assay Buffer. Pipette up and down to dissolve completely. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months. Keep on ice while in use.

9.6 NADPH Standard/NADPH Standard (lyophilized 0.2 µmol):

Reconstitute with 160 µL ddH_2O to generate 1.25 mM NADPH Standard solution. Aliquot NADPH standard so that you have enough volume to perform the desired number of assays. Store at –20°C. Keep on ice while in use.

9.7 PicoProbe I/PicoProbe in DMSO (400 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1-5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the probe is thawed, use within two months.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 12.5 µM NADPH working standard solution by adding 2 µL of 1.25 mM NADPH Standard to 198 µL of Assay Buffer.

10.2 Using 12.5 µM NADPH working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	NADPH 12.5 µM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount NADPH in well (pmol/well)
1	0	150	50	0
2	6	144	50	25
3	12	138	50	50
4	18	132	50	75
5	24	126	50	100
6	30	120	50	125

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- If using protease inhibitor, add to sample buffer immediately prior to use.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 1 – 5 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 200 µL Assay buffer (containing protease inhibitors).
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Keep on ice for 5 minutes.
11.1.6 Centrifuge sample for 5 minutes at 4°C at 10,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new tube.
11.1.8 Keep on ice.
11.1.9 Optional: quantify amount of protein in sample using a BCA Protein Assay Kit.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~ 50 – 100 mg).
11.2.2 Wash tissue in cold PBS.
11.2.3 Homogenize tissue in 200 µL Assay Buffer (containing protease inhibitors) with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.4 Centrifuge sample for 5 minutes at 4°C at 10,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.2.5 Collect supernatant and transfer to a new tube.
11.2.6 Keep on ice.
11.2.7 Optional: quantify amount of protein in sample using a BCA Protein Assay Kit.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 -10 µL sample [0.2 – 1 µg protein] (adjust volume to 50 µL/well with Assay Buffer).
- Sample Background Control wells = 2 - 10 µL samples [0.2 – 1 µg protein] (adjust volume to 50 µL/well with Assay Buffer).
- Positive control = 1 – 10 µL diluted Positive Control (adjust volume to 50 µL/well with Assay Buffer).

12.2 Reaction Mix:

12.2.1 Prepare 50 µL of Reaction and Background Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer V/PGM Assay Buffer	43	45
PicoProbe I/PicoProbe	1	1
Development Enzyme Mix IX/PGM Enzyme Mix	2	2
Developer IX/PGM Developer	2	2
PGM Substrate	2	0

12.2.2 Add 50 µL of Reaction Mix into each standard, sample and positive control well. Mix well.

12.2.3 Add 50 µl of Background Control Mix to Sample Background control well. Mix well.

12.3 Measurement:

12.3.1 Measure immediately fluorescence at Ex/Em = 535/587 nm in a microplate reader in kinetic mode for 10 – 60 minutes at 25ºC (room temperature).

Δ Note: Incubation time depends on the Phosphoglucomutase activity in the samples. Longer incubation may be required if activity in the sample is low. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the Phosphoglucomutase activity of the samples. The NADPH Standard Curve can be read in end point mode (ie. at the end of incubation time).

13. Calculations

- 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.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of Phosphoglucomutase activity in the sample:

13.2.1 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding fluorescence values at those points (RFU1 and RFU2).

13.2.2 Calculate ΔRFU for sample as follows:

$$\Delta RFU = RFU2 - RFU1$$

13.2.3 If sample background control reading is significant, subtract sample background control reading from sample reading.

13.2.4 Apply the ΔRFU to NADPH Standard Curve to get B pmol of NADPH generated during the reaction time.

13.2.5 PGM activity (pmol/min/µg or mU/mg) in the test samples is calculated as:

$$PGM\ Activity = \left(\frac{B}{\Delta T\ x\ M}\right) x\ D$$

Where:

B = amount of NADPH in the sample well calculated from Standard Curve (pmol)

ΔT = linear phase reaction time T2 – T1 (minutes)

M = amount of protein in sample well (µg)

D = sample dilution factor.

Unit definition:

1 Unit Phosphoglucomutase activity = amount of enzyme that will generate 1.0 µmol of NADPH per minute at pH8.0 at 25°C.

14. Typical Data

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

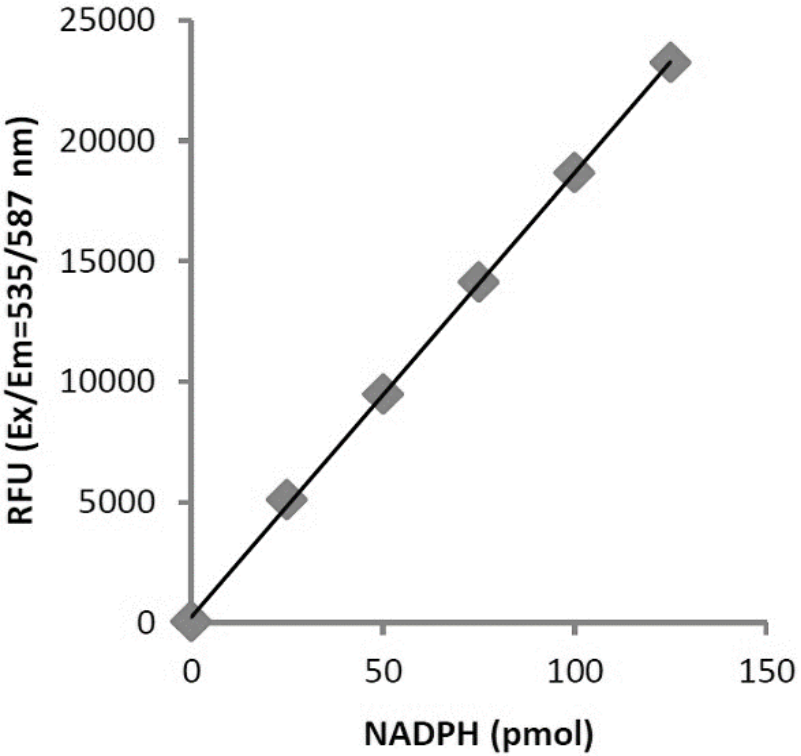


Figure 1. Typical NADPH standard calibration curve.

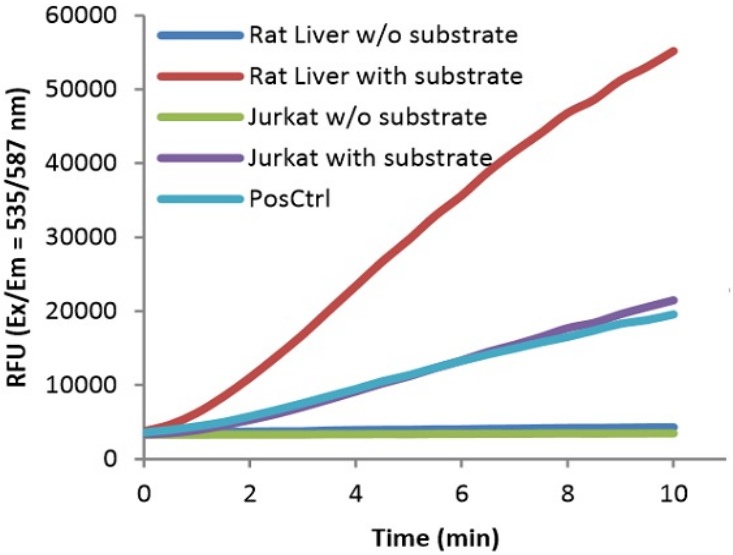


Figure 2. Kinetic curves showing PGM activity in positive control (PosCtrl, included in kit), Jurkat cell lysate (0.4 µg) and rat liver homogenate (0.2 µg). Curves of background control wells ("w/o substrate") are also shown.

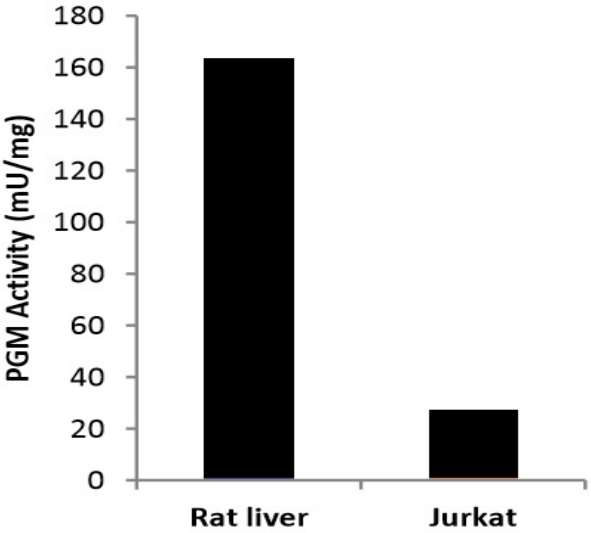


Figure 3. Typical Phosphoglucomutase specific activity in Jurkat cell lysate (0.4 µg) and rat liver homogenate (0.2 µg) calculated from data obtained from curves on figure 2.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; dilute positive control 1:1000; get equipment ready.
- Prepare NADPH standard dilution [25 – 125 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for Standard (50 µL), Sample Background control samples (50 µL), sample (50 µL) and positive control – diluted – wells (50 µL).
- Prepare a master mix for Reaction Mix and Background Control Mix:

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer V/PGM Assay Buffer	43	45
PicoProbe I/PicoProbe	1	1
Development Enzyme Mix IX/PGM Enzyme Mix	2	2
Developer IX/PGM Developer	2	2
PGM Substrate	2	0

- Add 50 µL Reaction mix to Standard, Sample and Positive control wells.
- Add 50 µl of Background Control Mix to Sample Background control well.
- Measure fluorescence immediately at Ex/Em = 535/587 nm in kinetic mode for 10 – 60 minutes at 25°C (RT).

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. 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)