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ab252890 Phosphoglycerate Kinase Activity Assay Kit (Colorimetric)

View kit datasheet: www.abcam.com/ab252890

(use www.abcam.cn/ab252890 for China, or www.abcam.co.jp/ab252890 for Japan)

For the measurement of Phosphoglycerate Kinase activity in a variety of samples.

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

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1. Overview

Phosphoglycerate Kinase Activity Assay Kit (Colorimetric) ab252890 provides a quick and easy way for monitoring PGK activity in various samples.

In the first step of this enzymatic assay, PGK converts 3-Phosphoglycerate and ATP to 1,3-Bisphosphoglycerate and ADP. The nascent intermediate is detected via a series of enzymatic reactions that lead the oxidation of NADH to NAD, which can be easily detected (OD= 340 nm).

The assay is simple, sensitive and can detect Phosphoglycerate Kinase Activity lower than 50 mU in variety of samples.

2. Protocol Summary

Prepare samples, standards, Background Control and Positive Control.

Prepare Reaction Mix and Background Control Mix.

Add 50 µL of Reaction Mix to each well containing the Positive Control and test samples.

Add 50 µL of Background Control mix to each well containing the Background Control sample.

Measure absorbance immediately at OD: 340 nm in kinetic mode for 5-60 mins at 37°C.

Calculate the sample Phosphoglycerate Kinase Activity.

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 4	25 mL	-20°C	-20°C
3-Phosphoglycerate	1 vial	-20°C	-20°C
ATP III	2 vials	-20°C	-20°C
PGK Developer	1 vial	-20°C	-20°C
NADH I	1 vial	-20°C	-20°C
PGK Positive Control	1 vial	-20°C	-20°C
96-Well UV Transparent Plate	1 unit	-20°C	-20°C

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and PGK Assay Buffer. The composition has not changed.

4. Materials Required, Not Supplied

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

- Dounce homogenizer
- Multi-well spectrophotometer
- Ammonium Sulfate Solution (Saturated, 4.32 M, ab273568)

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 3-Phosphoglycerate

Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

6.2 PGK Developer

Reconstitute with 220 µL dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

6.3 ATP III

Reconstitute each vial with 110 µL dH_2O. Pipette up and down to dissolve completely. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.

6.4 NADH I

Reconstitute with 480 µL dH_2O to generate 50 mM NADH I stock solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

6.5 PGK Positive Control

Reconstitute with 100 µL dH_2O and mix thoroughly. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.

7. Assay Procedure

Sample Preparation:

7.1 For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells (4 x 10^6) with 300 µL ice cold Assay Buffer 4, and place on ice for 10 mins.

7.2 Centrifuge at 10,000 *X g* for 5 mins, +4°C and collect the supernatant.

7.3 Use the ammonium sulfate precipitation method to remove small molecules that could cause interference: aliquot homogenates (110 µL) to a clean centrifuge tube, add saturated 4.32 M ammonium sulfate to 65% saturation (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate) mix and place on ice for 30 mins.

7.4 Spin down samples at 10,000 x *g* at +4°C for 10 mins, discard the supernatant, and resuspend the pellet back to the original volume with Assay Buffer 4.

7.5 Add 2-50 µL of each sample into two duplicate wells (Sample [S] and Sample Background Control [B]) of the 96-Well UV Transparent Plate; adjust final volume to 50 µL with Assay Buffer4.

7.6 For PGK Positive Control, dilute PGK 10-fold by adding 10 µL of PGK Positive Control to 90 µL Assay Buffer 4, mix well.

7.7 Add 2-20 µL of diluted Positive Control; adjust final volume to 50 µL with Assay Buffer 4.

Δ Note: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range.

Δ Note: To control for sample background, prepare parallel sample wells as sample background controls.

NADH Standard Curve:

7.8 Dilute NADH I 10-fold by adding 10 µL of 50 mM NADH to 90 µL of Assay Buffer 4 to make 5 mM NADH I. Mix well.

7.9 Add 0, 2, 4, 6, 8 and 10 µL of 5 mM NADH I into a series of wells of the 96-Well UV Transparent Plate (provided) to generate 0, 10, 20, 30, 40 and 50 nmol/well of NADH I.

7.10 Adjust volume to 100 µL/well with Assay Buffer 4.

Reaction Mix:

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

	Reaction Mix (µL)	**Background Control Mix* (µL)**
Assay Buffer 4	42.8	44.8
PGK Developer	2	2
ATP III	2	2
NADH I	1.2	1.2
3-Phosphoglycerate	2	---

Add 50 µL of the Reaction Mix to each well containing the Positive Control and test samples and 50 µl of Background Control mix to each well containing the Background Control sample. Mix well.
*For samples having high background, add 50 µL of Background Control Mix to each well and mix well.

Measurement:

7.12 Measure absorbance immediately at OD: 340 nm in kinetic mode for 5-60 mins at 37°C.
Δ Note: Incubation time depends on the phosphoglycerate kinase activity in the samples. We recommend kinetic measurement and choose two time points (t_1 and t_2) in the linear range to calculate the PGK activity of the samples. The NADH standard curve can be read in Endpoint mode (i.e., at the end of incubation time).

Calculation:

7.13 Subtract the 0 standard reading from all standard readings.

7.14 Plot the NADH standard curve.

7.15 Correct sample background by subtracting the value derived from the background control [B] from all sample readings [S].

7.16 Calculate the signal generated by PGK of the test sample:

Δ**OD = A_1 – A_2.**

7.17 Apply the ΔOD to the NADH standard curve to get P nmol of NADH oxidized by PGK during the reaction time ($\Delta t = t_2 - t_1$)**:**

Sample Phosphoglycerate Kinase Activity =

B/(Δt X V) x Dilution Factor = nmol/min/ml/ = mU/ml

Where:

B = NADH amount from standard curve (nmol).
Δ**t** = reaction time (min).
V = sample volume added into the reaction well (mL).

One unit of PGK is the amount of enzyme that generates 1.0 µmol of 1,3-Bisphosphoglycerate per min at pH 7.2 at 37°C.

8. Typical Data

Data provided **for demonstration purposes only**.

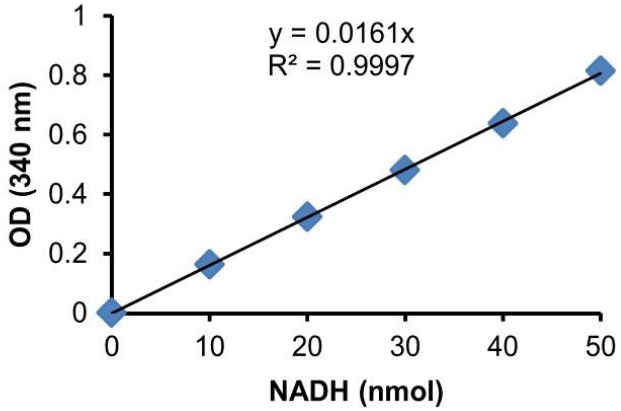

Figure 1. NADH standard curve.

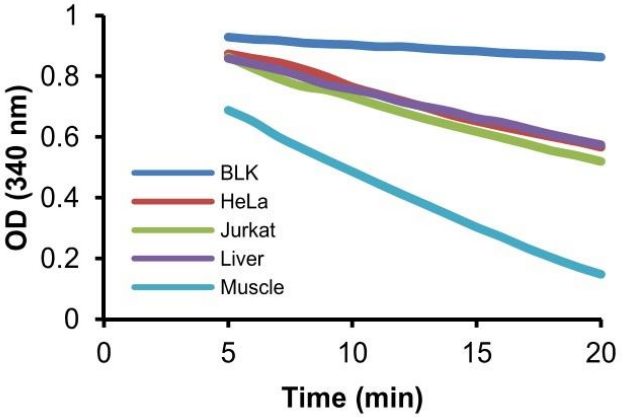

Figure 2. Kinetic measurement of Phosphoglycerate Kinase activity from various samples.

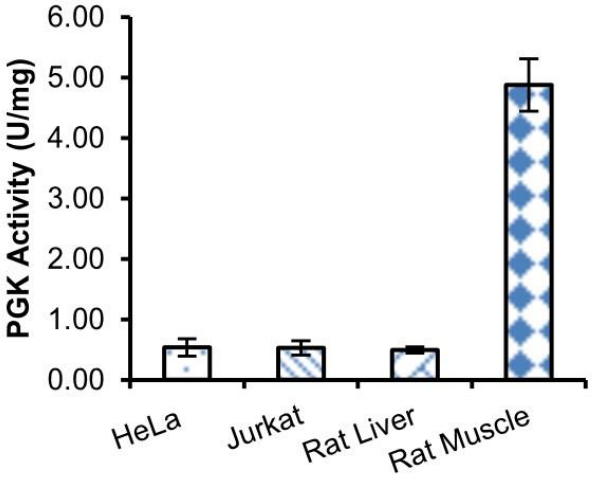

Figure 3. Relative PGK Activity was calculated in lysates prepared from rat liver (2 µg), rat muscle (0.5 µg), HeLa (Human epithelial cell line from cervix adenocarcinoma) (1.5 µg) and Jurkat (Human T cell leukemia cell line from peripheral blood) (1.5 µg).

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

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www.abcam.co.jp/contactus (Japan)