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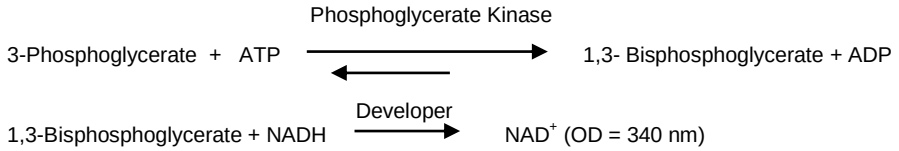
Phosphoglycerate Kinase Activity Assay Kit (Colorimetric)

Rev 07/19

(Catalog # K194-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphoglycerate Kinase (PGK1) (EC 2.7.2.3) participates in two of the most important biochemical pathways in living organisms: Glycolysis and Gluconeogenesis. PGK is responsible for the catalysis of the reversible reaction of 1,3-Bisphosphoglycerate (1,3-BPG) and ADP to 3-Phosphoglycerate (3-PG) and ATP. In humans, PGK1 and PGK2, two PGK isozymes have been characterized. While PGK1 is X-Chromosome-linked variant and expressed in all cells, PGK2 is autosome-linked variant and uniquely expressed in spermatogenic cells. Recent studies have shown that deficiency of PGK could lead to chronic hemolytic anemia, mental disorders and myopathy in humans, whereas PGK overexpression promotes gastric cancer cell invasiveness. Therefore, accurate measurement of PGK Activity is useful for mechanistic and possible diagnostic studies. BioVision's PGK Activity Assay kit 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.



II. Application:

- Measurement of Phosphoglycerate Kinase (PGK) Activity in various tissues/cells.
- Analysis of glycolytic Pathway and Gluconeogenesis Pathway.
- Mechanistic study for cancer development: e.g. Gastric Cancer.

III. Sample Type:

- Animal tissues: Muscle, Liver etc.
- Cell culture: HeLa, Jurkat, etc.

IV. Kit Contents:

Components	K194-100	Cap Code	Part Number
PGK Assay Buffer	25 ml	WM	K194-100-1
PGK Substrate	1 vial	Blue	K194-100-2
ATP	2 vials	Orange	K194-100-3
PGK Developer	1 vial	Green	K194-100-4
NADH	1 vial	Blue/Amber	K194-100-5
PGK Positive Control	1 vial	Purple	K194-100-6
U.V. Transparent Plate (96-well)	1 plate	-	K194-100-7

V. User Supplied Reagents and Equipment:

- Multi-well Spectrophotometer (Plate reader)
- Dounce Homogenizer
- Ammonium Sulfate Solution, BioVision Cat# 7096

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

- **PGK Assay Buffer:** Ready to use as supplied.
- **PGK Substrate and PGK Developer:** Reconstitute each vial 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.
- **ATP:** 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.
- **NADH:** Reconstitute with 480 µl dH_2O to generate **50 mM NADH** stock solution. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
- **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.

VII. Phosphoglycerate Kinase Assay Protocol:

1. **Sample Preparation:** For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells (4 x 10^6) with 300 µl ice cold PGK Assay Buffer, and place on ice for 10 minutes. Centrifuge at 10,000 X *g* for 5 min, 4°C and collect the supernatant. 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 (BioVision Cat # 7096) to 65% saturation (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate) mix and place on ice for 30 min. Spin down samples at 10,000 x *g* at 4°C for 10 min, discard the supernatant, and resuspend the pellet back to the original volume with PGK Assay Buffer.

Add 2-50 µl of each Sample into two duplicate wells of a U.V. Transparent Plate 96-well plate labeled as Sample and Sample Background Control. Adjust final volume to 50 µl/well with PGK Assay Buffer. For PGK Positive Control, dilute PGK 10-fold by adding 10 µl of PGK Positive Control to 90 µl PGK Assay Buffer, mix well. Add 2-20 µl of diluted Positive Control per well. Adjust the final volume to 50 µl/well with PGK Assay Buffer.

Notes:

a. For Unknown Samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. To control for Sample Background, prepare parallel Sample wells labeled as Sample Background Control.

2. **NADH Standard Curve:** Dilute NADH 10-fold by adding 10 µl of 50 mM NADH to 90 µl of PGK Assay Buffer to make 5 mM NADH Standard. Mix well. Add 0, 2, 4, 6, 8 and 10 µl of 5 mM NADH Standard into a series of wells of the U.V. Transparent Plateto generate 0, 10, 20, 30, 40 and 50 nmol/well of NADH Standard. Adjust volume to 100 µl/well with PGK Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Control Mix *
PGK Assay Buffer	42.8 µl	44.8 µl
PGK Developer	2 µl	2 µl
ATP	2 µl	2 µl
NADH Stock solution (50 mM)	1.2 µl	1.2 µl
PGK Substrate	2 µl	----

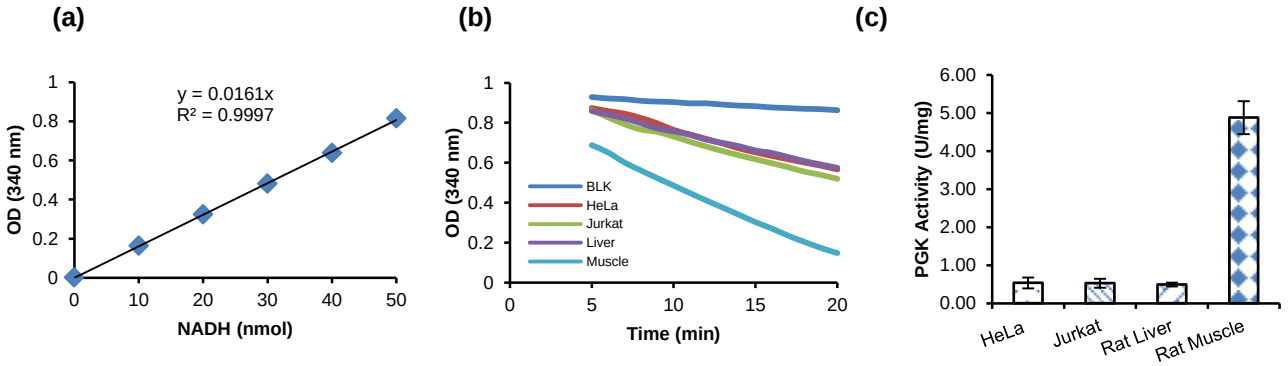
Add 50 µl of the Reaction Mix to each well containing the Positive Control and Sample(s) and 50 µl of Background Control mix to each well containing Sample Background Control. Mix well.

4. **Measurement:** Measure absorbance immediately at OD 340 nm in kinetic mode for 5-60 min at 37ºC.
Note: Incubation time depends on the PGK activity in the Samples. We recommend kinetic measurement and choose any two time points (t_1 & t_2) in the linear range to calculate the PGK activity of the Samples. The NADH Standard Curve can be read in Endpoint mode (at the end of incubation time).
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings. Plot the NADH Standard Curve. Correct Sample Background by subtracting the ΔOD of Sample Background Control from the ΔOD of Sample readings. Calculate the signal generated by PGK of the Sample by applying the ΔOD to the NADH Standard Curve to get B 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 the Standard curve (nmol).
Δt = 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 37ºC.



Figures: (a) NADH Standard Curve. (b) Kinetic measurement of PGK activity in various Samples. BLK represents the Reagent Control well containing 50 µl PGK Assay Buffer and 50 µl Reaction Mix. (C) Relative PGK Activity was calculated in lysates prepared from Rat Liver (2 µg), Rat Muscle (0.5 µg), HeLa (1.5 µg) and Jurkat (1.5 µg). Assays were performed following the kit protocol.

VIII. Related Products:

Glucose and Sucrose Assay Kit (K616)
Glucose Uptake Colorimetric Assay Kit (K676)
Glucose Uptake Fluorometric Assay Kit (K666)
NAD/NADH Quantification Kit (K337)
PicoProbe™ Glucose-6-Phosphate Assay Kit (K687)
Phosphoglucomutase Assay Kit (K774)
Pyruvate Colorimetric/Fluorometric Kit (K609)
Glucose-6-Phosphate Dehydrogenase Assay Kit (K757)
Hexokinase Colorimetric Assay kit (K789)
Hexokinase Assay Kit (K789)
Glucose Dehydrogenase Activity Assay Kit (K679)
GAPDH Activity Assay Kit (K680)

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