CREATIVE BIOMART®
Assay Kit

Phosphoglycerate Kinase Activity Assay Kit (Colorimetric)

Product Information

Short Name

Phosphoglycerate Kinase Activity Assay Kit (Colorimetric)

Cat.No. KITZ-009

Size

100 tests

Product Overview

Phosphoglycerate Kinase Activity Assay Kit (Colorimetric) 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 50mU in variety of samples.

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.

Shipping

Gel pack

Kit Components

PGK Assay Buffer: 25 mL -20°C
PGK Substrate: 1 vial -20°C
ATP: 2 vials -20°C
PGK Developer: 1 vial -20°C
NADH: 1 vial -20°C
PGK Positive Control: 1 vial -20°C

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U.V. Transparent Plate (96-well): 1 unit -20°C

Materials Required but 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, 4.1 M)

Detection method Colorimetric, OD = 340 nm

Assay Protocol

Assay protocol:
1. Reagent Preparation
Briefly centrifuge small vials at low speed prior to opening.
1.1 PGK Substrate
Reconstitute with 220µL dH2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
1.2 PGK Developer
Reconstitute with 220µL dH2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
1.3 ATP
Reconstitute each vial with 110µL dH2O. 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.
1.4 NADH
Reconstitute with 480µL dH2O to generate 50 mM NADH stock solution. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.
1.5 PGK Positive Control
Reconstitute with 100µL dH2O and mix thoroughly. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.
2. Sample Preparation:
2.1 For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells (4 x 106) with 300 µL ice

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cold PGK Assay Buffer, and place on ice for 10 mins.
2.2 Centrifuge at 10,000 X g for 5 mins, +4°C and collect the supernatant.
2.3 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.
2.4 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 PGK Assay Buffer.
2.5 Add 2-50µL of each sample into two duplicate wells (Sample [S] and Sample Background Control [B]) of the U.V. Transparent Plate 96 well plate; adjust final volume to 50 µL with PGK Assay Buffer.
2.6 For PGK Positive Control, dilute PGK 10-fold by adding 10µL of PGK Positive Control to 90µL PGK Assay Buffer, mix well.
2.7 Add 2-20µL of diluted Positive Control; adjust final volume to 50 µL with PGK Assay Buffer.
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.
3. NADH Standard Curve:
3.1 Dilute NADH 10-fold by adding 10µL of 50mM NADH to 90µL of PGK Assay Buffer to make 5 mM NADH Standard. Mix well.
3.2 Add 0, 2, 4, 6, 8 and 10µL of 5 mM NADH Standard into a series of wells of the U.V. Transparent Plate (provided) to generate 0, 10, 20, 30, 40 and 50 nmol/well of NADH Standard.
3.3 Adjust volume to 100µL/well with Assay Buffer.
4. Reaction Mix:
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)
PGK Assay Buffer	42.8	44.8
PGK Developer	2	2

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ATP 2 2
NADH (50 mM) 1.2 1.2
PGK Substrate 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.
5. Measurement:
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 (t1 and t2) 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).
6. Calculation:
6.1 Subtract the 0 standard reading from all standard readings.
6.2 Plot the NADH standard curve.
6.3 Correct sample background by subtracting the value derived from the background control [B] from all sample readings [S].
6.4 Calculate the signal generated by PGK of the test sample: ΔOD = A1 – A2.
6.5 Apply the ΔOD to the NADH standard curve to get P nmol of NADH oxidized by PGK during the reaction time (Δt = t2 – t1):
Sample Phosphoglycerate Kinase Activity = B/(Δt X V) x Dilution Factor = nmol/min/ml/ = mU/ml
Where:
B = NADH amount from 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 37°C.

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