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# 2-Phosphoglycerate Colorimetric/Fluorometric Assay Kit

*rev. 4/13*

**(Catalog # K778-100; 100 assays; Store at -20°C)**

## I. Introduction:

2-phosphoglycerate (2PG) is an important intermediate in the glycolysis pathway. 2-Phosphoglycerate is converted by enolase to phosphoenolpyruvate (PEP) which is a key step from glucose to pyruvate. Aberrant glycolytic metabolism is a highly studied and potentially critical mechanism for ATP generation in cancer cells (The Warburg effect). Measurement of intracellular 2PG levels is a useful tool for analyzing the glycolytic pathway and its relevance to cancer research. BioVision's 2-Phosphoglycerate Assay kit is a sensitive, fast and easy-to-use kit. In this assay, 2PG is converted by Enzyme Mix to PEP, which is further converted to pyruvate. The pyruvate is oxidized to generate color (OD 570 nm) and fluorescence (Ex/Em = 535/587 nm). The colored product or fluorescence intensity is proportional to 2PG level. This assay kit can detect 2PG level below 20 pmol and can be used for a variety of sample types.

$$\text{2-Phosphoglycerate} \xrightarrow{\text{Enzyme Mix}} \text{Phosphoenolpyruvate} + H_2O$$

$$\text{Phosphoenolpyruvate} \xrightarrow{\text{Converter}} \text{Pyruvate} \xrightarrow{\text{Developer}} \text{Color (OD 570 nm) or Fluorescence (Ex/Em = 535/587 nm)}$$

## II. Application:

- Measurement of 2-Phosphoglycerate in various tissues/cells
- Analysis of carbohydrate metabolism and cell signaling
- Cancer research

## III. Sample Type:

- Animal tissues: e.g. liver, muscle and heart etc.
- Cell culture: Adherent or suspension cells

## IV. Kit Contents:

| Components | K778-100 | Cap Code | Part Number |
|---|---|---|---|
| 2PG Assay Buffer | 25 ml | WM | K778-100-1 |
| 2PG Probe (in DMSO) | 0.2 ml | Red | K778-100-2A |
| 2PG Enzyme Mix (Lyophilized) | 1 vial | Blue | K778-100-3 |
| 2PG Converter (Lyophilized) | 1 vial | Purple | K778-100-4 |
| 2PG Developer (Lyophilized) | 1 vial | Green | K778-100-5 |
| 2PG Standard (Lyophilized) | 1 vial | Yellow | K778-100-6 |

## V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom (for Colorimetric Assay)
- 96-well white plate with flat bottom (for Fluorometric Assay)
- Multi-well spectrophotometer (ELISA reader)

## VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm 2PG Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

## VII. Reagent Preparation and Storage Conditions:

- **2PG Probe**: Ready to use as supplied. Warm 1-2 min at 37ºC to melt the frozen DMSO before use. Mix well, store at -20ºC, protect from light and moisture. Use within two months.
- **2PG Enzyme Mix, 2PG Converter and 2PG Developer**: Reconstitute with 220 µl 2PG Assay Buffer. Pipette up and down to dissolve completely. Keep on ice while in use. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Stable for 2 months at -20ºC.
- **2PG Standard:** Reconstitute with 100 µl $dH_2O$ to generate 100 mM (100 nmol/µl) 2PG Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

## VIII. 2PG Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly. Rapidly homogenize tissue (10 mg) or cells (1 x $10^6$) with 200 µl ice cold 2PG Assay Buffer on ice. Centrifuge at 12000 rpm for 5 minutes to remove cell debris and save the supernatant. Add 1-50 µl samples into a 96 well plate and bring the volume to 50 µl with 2PG Assay Buffer.

   **Notes:**

   **A.** For unknown samples, we suggest testing several doses of your samples to ensure the readings are within the Standard Curve range.

   **B.** Pyruvate in samples will generate background. For samples having high pyruvate levels, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:**

   **For Colorimetric Assay:** Dilute 2PG Standard to 1 mM by adding 10 µl of 100 mM 2PG Standard to 990 µl $dH_2O$, mix well. Add 0, 2, 4, 6, 8 & 10 µl of 1 mM 2PG Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol/well 2PG Standard. Adjust volume to 50 µl/well with 2PG Assay Buffer.

**For Fluorometric Assay:** Dilute 2PG to 0.025 mM by adding 25 µl of 1 mM 2PG Standard to 975 µl dH2O, mix well. Add 0, 2, 4, 6, 8 & 10 µl of 0.025 mM (0.025 nmol/ul) 2PG Standard into a series of wells in 96 well plate to generate 0, 50, 100, 150, 200, and 250 pmol/well. Adjust volume to 50 µl/well with 2PG Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

| | Reaction Mix | Background Control Mix |
|---|---|---|
| 2PG Assay Buffer | 42 µl | 44 µl |
| 2PG Probe** | 2 µl | 2 µl |
| 2PG Enzyme Mix | 2 µl | ---- |
| 2PG Converter | 2 µl | 2 µl |
| 2PG Developer | 2 µl | 2 µl |

Add 50 µl of the Reaction Mix to each well containing the Standard and test samples & 50 µl of Background Control Mix to sample background control well(s). Mix well.
****Note:** For fluorometric Assay, use 1/10 of Probe (0.2 µl/well) to reduce the background.

4. **Measurement:** Incubate at room temperature for 40 minutes. Measure $OD_{570nm}$ or fluorescence (Ex/Em = 535/587 nm).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the 2PG Standard Curve. If the sample background control reading is significantly high, subtract the sample background reading from sample reading. Apply the corrected sample reading to the 2PG Standard Curve to get B nmol or pmol 2PG in the sample wells.

**Sample 2PG Concentration (C) = B/V x Dilution Factor = nmol/µl = µmol/ml = mM**

Where: **B =** the amount of 2PG in the sample well (nmol/pmol)
**V =** the sample volume used in the reaction well (µl)

2PG in samples can also be expressed in nmol/mg of protein
2-Phosphoglycerate molecular weight: 186.06 g/mol

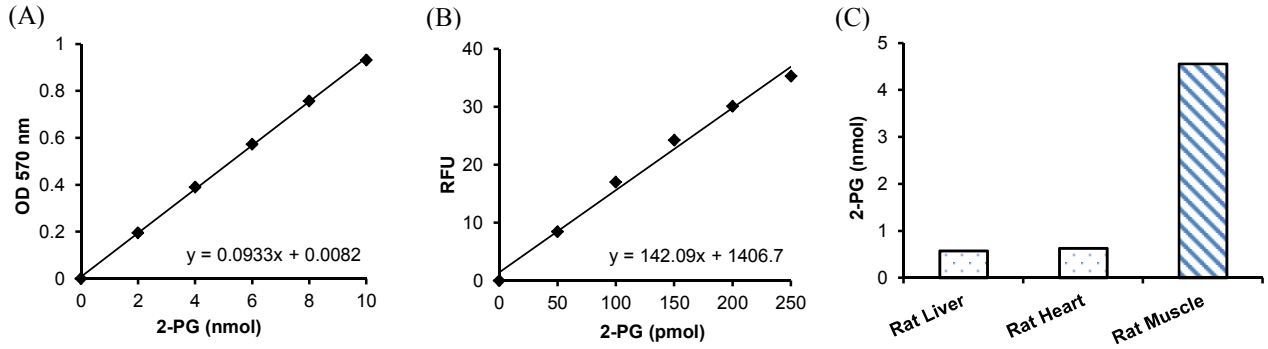


**Figure:** 2PG Standard Curve: (A) Colorimetric and (B) Fluorometric. (C) Measurement of 2PG level in rat liver, heart and muscle lysate (200 µg protein each). Assays were performed according to kit protocol.

## IX. RELATED PRODUCTS:

Glucose Colorimetric/Fluorometric Assay kit
PicoProbe™ Glucose Fluorometric Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Glucose-1-Phosphate Colorimetric Assay Kit
Glucose Uptake Colorimetric Assay Kit
Galactose Colorimetric/Fluorometric Assay Kit
Glycogen Colorimetric/Fluorometric Assay Kit
Hexokinase Colorimetric Assay Kit
Maltose & Glucose Colorimetric/Fluorometric Assay Kit
PEP Colorimetric/Fluorometric Assay Kit
Phosphoglucomutase Colorimetric Assay Kit
Pyruvate Colorimetric/Fluorometric Assay Kit
Maltose and Glucose Assay Kit
NADP/NADPH Quantification Kit
Starch Assay Kit
ATP Colorimetric/Fluorometric Assay Kit
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit
Glucose Colorimetric Assay Kit II
Glucose-6-Phosphate Dehydrogenase Assay Kit
PicoProbe™ Glucose-6-Phosphate Fluorometric Assay Kit
Glucose Uptake Fluorometric Assay Kit
Galactose & Lactose Colorimetric/Fluorometric Assay Kit
Glycogen Colorimetric Assay Kit II
Maltose Colorimetric/Fluorometric Assay Kit
PicoProbe™ Lactate Fluorometric Assay Kit
Phosphofructokinase (PFK) Activity Colorimetric Assay Kit
Phosphoglucose Isomerase Colorimetric Assay Kit
Pyruvate Kinase Activity Colorimetric Assay Kit
NAD/NADH Quantification Kit
PicoProbe™ NADH Fluorometric Assay Kit
Total Carbohydrate Assay Kit

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