Tribioscience

2-Phosphoglycerate Colorimetric/Fluorometric Assay
(TBS2206; 100Assays; Store at -20°C)

DESCRIPTION

2-phosphoglycerate (2PG) is an important intermediate in the glycolysis pathway. 2PG 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.

Tribioscience's 2-Phosphoglycerate Assay kit provides a sensitive, fast and easy-to-use approach. 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.

FEATURES

- **Flexible:** Can be used 96 well or 384 well plate with colorimetric and fluorometric assay.
- **Accuracy**: Absorbance and Fluorescence intensity is proportional to the 2PG level.

Kit Components and Storage for 100 tests

Component	Part Size	Part Number
2PG Assay Buffer	15 mL	TBS2206-01
2PG Probe	0.25 mL	TBS2206-02
2PG Enzyme Mix	0.25 mL	TBS2206-03
2PG Converter	0.25 mL	TBS2206-04
2PG Developer	0.25 mL	TBS2206-05
2PG Standard (100mM)	50 µL	TBS2206-06
Store at -20°C for 1 year after receipt		

APPLICATIONS

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

DIRECTIONS FOR USE

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 concentrations 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.

1. Standard Curve Preparation:

For Colorimetric Assay: Dilute 2PG Standard to 1 mM by adding 2 µl of 100 mM 2PG Standard to 198 µl Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10, 20 µL of 1 mM 2PG Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8, 10, and 20 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 2.5 µl of 1 mM 2PG Standard to 97.5 µl Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10, and 20 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, 250, and500 pmol/well. Adjust volume to 50 µl/well with 2PG Assay Buffer.

2. 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:**

Component	Reaction Mix	Background Control Mix
2PG Assay Buffer	42 µL	44 µL
2PG Probe	2 µL	2 µL
2PG Enzyme Mix	2 µL	xx
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, and 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.

3. Measurement: Incubate at room temperature for 30-60 minutes. Measure OD value at 570nm or fluorescence (Ex/Em = 535/587 nm).

4. 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.

TYPICAL DATA

Fig.1 2PG Colorimetric Assay

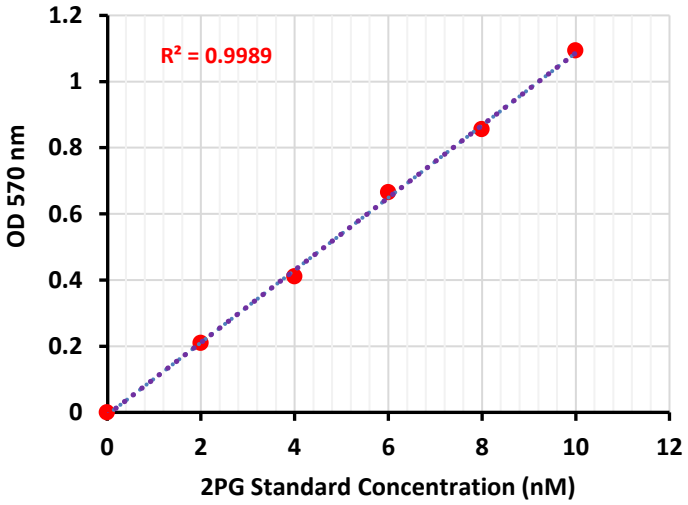
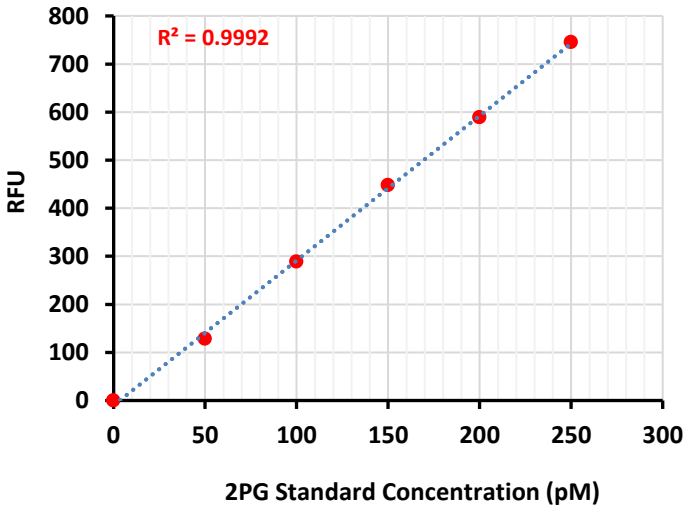


Fig. 2 2PG Fluorometric Assay



RELATED PRODUCTS

Resazurin Cell Viability (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
MTT Cell Viability Assay (TBS2003)
MTS Cell Viability Assay (TBS2004)
Catalase Assay (TBS2006)
ATP Colorimetric/ Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay (TBS3020)
XTT Cell Viability Assay (TBS2021)
Caspase-3 Colorimetric Assay (TBS2030)
BrdU Cell Proliferation Colorimetric Assay (TBS2086)
Cytochrome c Reductase Activity Assay (TBS2116)
AOPI Viability Assay for Flow Cytometry (TBS2069)

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