

Pyruvate Assay Kit

(Catalog #K609-100; 100 assays; Store kit at -20°C)

I. Introduction:

Pyruvate is a central molecule in metabolism through which sugars enter the citric acid cycle. Pyruvate can be converted to carbohydrates during gluconeogenesis or to fatty acids via acetyl CoA. High levels of pyruvate are associated with liver disease and genetic disorders. Pyruvate has also been used to stimulate metabolism leading to loss of body weight. BioVision provides a simple, direct and automation-ready procedure for measuring pyruvate concentration in various biological samples such as blood, cells, culture and fermentation media, etc. In the assay, pyruvate is oxidized by pyruvate oxidase via enzyme reactions to generate color (at $\lambda = 570$ nm) and fluorescence (at Ex/Em = 535/587 nm). Since the color or fluorescence intensity is proportional to pyruvate content, the pyruvate concentration can be accurately measured. The kit detects 1-10000 μ M pyruvate.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Pyruvate Assay Buffer	25 ml	WM	K609-100-1
Pyruvate Probe	Lyophilized	Red	K609-100-2
DMSO (anhydrous)	400 μ l	Brown	K609-100-3
Pyruvate Enzyme Mix	Lyophilized	Green	K609-100-4
Pyruvate Standard (100 nmol/ μ l)	100 μ l	Yellow	K609-100-5

III. Reagent Preparation and Storage Conditions:

Pyruvate Probe: Dissolve with 220 μ l of DMSO (provided, need to warm up >18°C to become liquid) before use. Mix well, store at -20°C, protect from light and moisture. Use within two months.

Pyruvate Enzyme Mix: Dissolve with 220 μ l Pyruvate Assay Buffer. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.

IV. Pyruvate Assay Protocol:

1. Standard Curve Preparations:

Colorimetric assay: Dilute the Pyruvate Standard to 1 nmol/ μ l by adding 10 μ l of the Standard to 990 μ l of Pyruvate Assay Buffer, mix well.

Fluorometric assay: Dilute the Pyruvate Standard to 1 nmol/ μ l as for the colorimetric assay. Then dilute the standard another 10-fold to 0.1 nmol/ μ l by taking 10 μ l into 90 μ l of Pyruvate Assay Buffer. Mix well.

Add 0, 2, 4, 6, 8, 10 μ l into a series of standards wells. Adjust volume to 50 μ l/well with Pyruvate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Pyruvate Standard for the colorimetric assay (0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well for the fluorometric assay).

- Sample Preparations:** Prepare test samples in 50 μ l/well with Pyruvate Assay Buffer in a 96-well plate. Serum can be directly added into sample wells, and adjust volume to 50 μ l/well with Pyruvate Assay Buffer (serum contains ~50-100 pmol/ μ l pyruvate). Tissues or cells can be extracted with 4 volume of the Pyruvate Assay Buffer, centrifuge to get clear pyruvate extract. We suggest using several doses of your sample to ensure the readings are within the standard curve range. Due to the presence of LDH in serum, care must be taken during sample processing to prevent the conversion of pyruvate to lactate. Samples can be deproteinized by 10Kd cutoff spin filter (BioVision Cat #1997-25) to remove proteins.

- Reaction Mix Preparation:** Mix enough reagents for the number of assays performed. For each well, prepare a total 50 μ l Reaction Mix containing the following components. Mix well before use:

46 μ l Pyruvate Assay Buffer
2 μ l Pyruvate Probe
2 μ l Enzyme Mix

- Add 50 μ l of the Reaction Mix to each well containing the Pyruvate Standard or test samples, mix well.
- Incubate the reaction for 30 minutes at room temperature, protect from light.
- Measure O.D. 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.
- Calculation:** Correct background by subtracting the value derived from the 0 pyruvate control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot standard curve nmol/well vs. O.D. 570 nm readings. Then apply the sample readings to the standard curve to get pyruvate amount in the sample wells (Py).

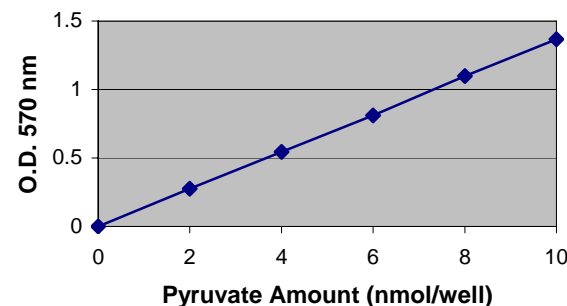
The pyruvate concentrations in the test samples:

$$C = \text{Py}/\text{Sv} \text{ (nmol}/\mu\text{l or mM)}$$

Where: Py is the amount of pyruvate (nmol) of your sample from standard curve. Sv is the sample volume (μ l) added into the sample well.

Pyruvate molecular weight: 88.08. Pyruvate concentration in your sample can be expressed as nmol/ml, or mg/ml, or mg/dL or mM (mmol/liter).

$$1 \text{ mM} = 8.81 \text{ mg/dL}$$



V. Related Products:

- Lactate Assay Kit
- Cholesterol Assay Kit
- Glutathione Assay Kit
- Glucose, Sucrose Assay Kit
- Maltose Assay Kit
- Ascorbic acid Assay Kit
- Free Fatty Acid Assay Kit
- NAD(P)/NAD(P)H Assay Kit
- ATP/ADP Assay Kit
- Cell Proliferation Assay Kit
- Cytotoxicity Assay Kit