Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Pyruvate Colorimetric/Fluorometric Assay Kit

rev. 12/14

(Catalog # K609-100; 100 assays; Store 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 (OD 570 nm) and fluorescence (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 µM to 10 mM pyruvate.

II. Application:

- Measurement of pyruvate in various tissues/cells/serum/saliva etc.
- Analysis of metabolism in various cells

III. Sample Type:

- Animal tissues
- Cell culture: adherent or suspension cells
- Serum, saliva

IV. Kit Contents:

Components	K609-100	Cap Code	Part Number
Pyruvate Assay Buffer	25 ml	WM	K609-100-1
Pyruvate Probe (in DMSO)	200 µl	Red	K609-100-2A
Pyruvate Enzyme Mix	Lyophilized	Green	K609-100-4
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K609-100-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Pyruvate Assay Buffer:** Warm to room temperature before use. Store at 4˚C or -20˚C.
- **Pyruvate Probe:** Briefly warm at 37˚C for 1-2 min. to completely melt the DMSO solution. Mix well, store at –20˚C, protected from light and moisture. Use within two months.
- **Pyruvate Enzyme Mix**: Dissolve with 220 µl Pyruvate Assay Buffer. Pipette up and down to completely dissolve. Store at –20˚C. Use within two months.

VIII. Pyruvate Assay Protocol:

1. **Sample Preparation:** Serum can be directly added into sample wells (serum contains ~50-100 pmol/µl pyruvate). Centrifuge saliva at 10,000 x g for 10 min. at 4°C. Add 10-20 µl into 96-well plate. Adjust volume to 50 µl/well with Pyruvate Assay Buffer. Tissues or cells can be extracted with 4 volume of the Pyruvate Assay Buffer. Centrifuge (10000 x g; 10 min.; 4°C) to remove insoluble material. Collect supernatant. Add 2-50 µl samples into 96-well plate. Adjust the volume to 50 µl/well with Pyruvate Assay Buffer. Samples should be stored at -80°C if assayed later.

   Notes:

   a. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

   b. Due to the presence of LDH in serum, care must be taken during sample processing to prevent the conversion of pyruvate to lactate.

   c. Samples can be deproteinized by 10K Spin Column (Cat. # 1997) to remove proteins that consume pyruvate.

   d. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.

   e. Endogenous compounds may interfere with the reaction. To ensure accurate determination of Pyruvate in the test samples, we recommend spiking samples with a known amount of Standard (6 nmol).

2. **Standard Curve Preparation:** For colorimetric assay: dilute the Pyruvate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of Pyruvate Assay Buffer, mix well. For fluorometric assay: Dilute the Pyruvate Standard to 1 nmol/µl as for the colorimetric assay. Then further dilute the Standard to 0.1 nmol/µl by adding 10 µl of 1 nmol/µl Standard into 90 µl of Pyruvate Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells in 96-well plate. Adjust the volume to 50 µl/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).

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing the following components. Mix well before use:

	Reaction Mix	*Background Control Mix
Pyruvate Assay Buffer	46 µl	48 µl
Pyruvate Probe**	2 µl	2 µl
Enzyme Mix	2 µl	---

Add 50 µl of the Reaction Mix to each well containing the Pyruvate Standard & test samples, mix well.

* For samples having background, add 50 µl of Background Control Mix to sample background control well(s).

** For fluorometric assay use 0.4 µl Pyruvate Probe and 47.6 µl Pyruvate assay buffer to reduce background.

4. **Measurement:** Incubate the reaction for 30 min. at room temperature. Protect from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/590 nm) in a microplate reader.
5. **Calculation:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample readings. Plot the Pyruvate Standard Curve. For unspiked samples, apply the corrected absorbance or fluorescence to the Pyruvate Standard Curve to get B nmol of Pyruvate in the sample well.

Sample Pyruvate concentration (C) = B/V X D (nmol/µl or mM)

Where: **B** is the amount of pyruvate in the sample well (nmol)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Pyruvate amount in sample well (B) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + Pyruvate Std(corrected)}})-(\mathrm{OD}_{\text{sample(corrected)}})}\right) * Pyruvate\ Spike\ (\mathrm{pmol})$

Pyruvate molecular weight: 88.08.
Pyruvate concentration in your samples can be expressed as nmol/ml, or mg/ml, or mg/dL or mM (mmol/liter). 1 mM = 8.81 mg/dl.

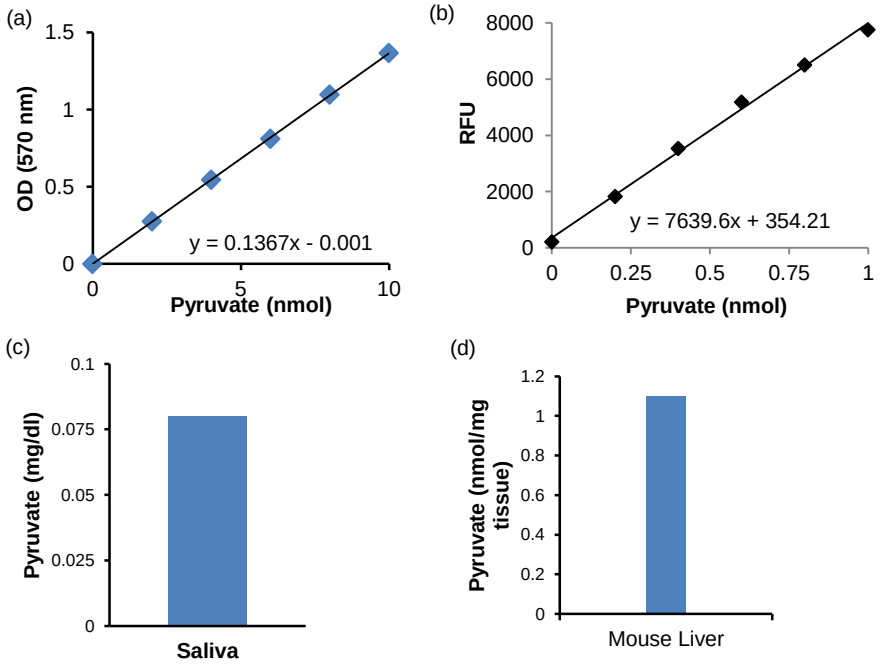

Figure: Pyruvate Standard Curve: colorimetric (a), fluorometric (b). Quantitation of pyruvate in human saliva (c) and mouse liver lysate (d). Saliva sample was centrifuged at 10,000 x g for 10 min. at 4°C. Supernatant (10 µl) was spiked with known amounts of pyruvate Standard (6 nmol) and assayed according to kit protocol. Mouse liver homogenate was centrifuged and supernatant was collected. Supernatant was deproteinized using 10 kDa spin column (Cat. # 1997) and filtrate was diluted 5 fold. 10 ul of this diluted filtrate was spiked with known amount of pyruvate Standard (6 nmol) and assayed according to kit protocol.

IX. RELATED PRODUCTS:

Pyruvate Kinase Activity Colorimetric/Fluorometric Assay Kit (K709)
Alpha-Ketoglutarate Colorimetric/Fluorometric Assay Kit (K677)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Isocitrate Dehydrogenase Activity Colorimetric Assay Kit (K756)
PEP Colorimetric/Fluorometric Assay Kit (K365)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Isocitrate Colorimetric Assay Kit (K656)
Fumarate Colorimetric Assay Kit (K633)

FOR RESEARCH USE ONLY! Not to be used on humans.