EnzyChrom™ Pyruvate Assay Kit (Cat# EPYR-100)

Quantitative Colorimetric/Fluorimetric Pyruvate Determination

DESCRIPTION

PYRUVATE is a key intermediate in cellular metabolic pathways. Pyruvate can be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine and to ethanol. Abnormal levels of pyruvate have been linked to liver diseases and metabolic disorders. Simple, direct and automation-ready procedures for measuring pyruvate concentrations find wide applications in research and drug discovery. BioAssay Systems' pyruvate assay uses a single Working Reagent that combines pyruvate oxidase and hydrogen peroxide determination in one step. The color intensity of the reaction product at 570nm or fluorescence intensity at λem/ex = 585/530nm is directly proportional to pyruvate concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 2 to 500 µM (17 µg/dL to 4.4 mg/dL) pyruvate for colorimetric assays and 0.2 to 50 µM for fluorimetric assays.
Simple and convenient. The procedure involves addition of a single working reagent and incubation for 30 min at room temperature, compatible for HTS assays.
Improved reagent stability. The optimized formulation has greatly enhanced the reagent and signal stability.

APPLICATIONS:

Direct Assays: pyruvate in biological samples.
Drug Discovery/Pharmacology: effects of drugs on pyruvate metabolism.

KIT CONTENTS

Enzyme Mix: 10 mL
Dye Reagent: 120 µL
Standard: 400 µL 25 mM Pyruvate

Storage conditions. The kit is shipped on dry ice. Store all reagents at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIEMTRIC PROCEDURE

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to room temperature. Prepare a 500 µM Standard Premix by mixing 10 µL of the 25 mM Standard and 490 µL H_2O. Dilute Standard in distilled water as follows.

No	Premix + H_2O	Vol (µL)	Pyruvate (µM)
1	100µL + 0µL	100	500
2	80µL + 20µL	100	400
3	60µL + 40µL	100	300
4	40µL + 60µL	100	200
5	30µL + 70µL	100	150
6	20µL + 80µL	100	100
7	10µL + 90µL	100	50
8	0µL + 100µL	100	0

   Transfer 10 µL standards and 10 µL samples into separate wells of a clear flat-bottom 96-well plate.

2. For each reaction well, mix 94 µL Enzyme Mix and 1 µL Dye Reagent in a clean tube. Transfer 90 µL Working Reagent into each assay well. Tap plate to mix. Freeze unused reagents for future use.

3. Incubate 30 min at room temperature. Read optical density at 570nm (550-585nm).

Note: if the Sample OD is higher than the Standard OD at 500 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

CALCULATION

Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The pyruvate concentration of Sample is calculated as

$$[\text{Pyruvate}] = \frac{OD_{SAMPLE} - OD_{H2O}}{\text{Slope}} \quad (\mu M)$$

OD_{SAMPLE} and OD_{H2O} are optical density values of the sample and water.

Conversions: 1mM pyruvate equals 8.7 mg/dL or 87 ppm.

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 0.2 to 50 µM pyruvate. Dilute the Standards prepared in Colorimetric Procedure 1:10 in H_2O.

Transfer 10 µL standards and 10 µL samples into separate wells of a *black* 96-well plate.

Add 90 µL Working Reagent (see *Colorimetric Procedure*). Tap plate to mix.

Incubate 30 min at room temperature and read fluorescence at λ_{ex} = 530nm and λ_{em} = 585nm.

If assays in 384-well plate are desired, use 5µL Standards and 45 µL Working Reagent.

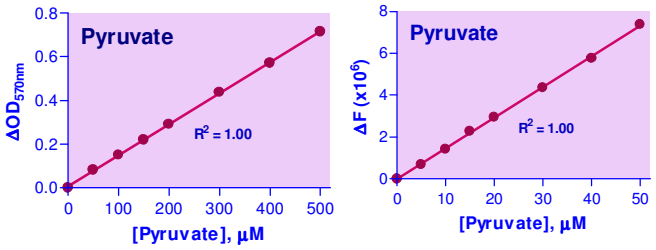
The pyruvate concentration of Sample is calculated as

$$[\text{Pyruvate}] = \frac{F_{SAMPLE} - F_{H2O}}{\text{Slope}} \quad (\mu M)$$

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well or 384-well plates (e.g. Corning Costar) and plate reader.

Pyruvate Standard Curves



96-well colorimetric assay ***384-well fluorimetric assay***

LITERATURE

1. Hansen JL, Freier EF. (1978). Direct assays of lactate, pyruvate, beta-hydroxybutyrate, and acetoacetate with a centrifugal analyzer. Clin Chem. 24(3):475-9.

2. Sutherland DV, Barns AM, Ross CA. (1995). Trypanosoma evansi: measurement of pyruvate production as an indicator of the drug sensitivity of isolates in vitro. Trop Med Parasitol. 46(2):93-8.

3. Chariot P. et al (1994). Optimal handling of blood samples for routine measurement of lactate and pyruvate. Arch Pathol Lab Med. 118(7):695-7.