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Product Information

Pyruvate Assay Kit

Catalog Number **MAK332**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product 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. The Pyruvate Assay Kit uses a single Reaction Mix that combines pyruvate oxidase and hydrogen peroxide determination in one step. The color intensity of the reaction product at 570 nm or fluorescence intensity at λ_{ex} = 530 nm/λ_{em} = 585 nm is directly proportional to the pyruvate concentration in the sample.

The Pyruvate Assay Kit has a linear detection range for pyruvate in a 96 well plate assay of 2–500 μM (17 μg/dL to 4.4 mg/dL) for colorimetric assays and 0.2–50 μM for fluorometric assays.

Suitable for pyruvate detection in biological samples and for studying the effects of drugs on pyruvate metabolism.

Components
The kit is sufficient for 100 colorimetric or fluorometric assays in 96 well plates.

Component	Amount
Enzyme Mix Catalog Number MAK332A	10 mL
Dye Reagent Catalog Number MAK332B	120 μL
Standard (25 mM Pyruvate) Catalog Number MAK332C	400 μL

Reagents and Equipment Required but Not Provided.
- Pipetting devices and accessories (e.g., multichannel pipettor)
- Centrifuge tubes
- 96 well flat bottom plates– It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on dry ice. Store all components at –20 °C upon receiving.

Procedures

Equilibrate all components to room temperature prior to assay.

Sample Preparation

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

Standard Preparation for Colorimetric Procedure

Prepare a 500 μM Standard Premix by mixing 10 μL of the 25 mM Standard and 490 μL of ultrapure water. Dilute 500 μM Standard Premix according to Table 1.

Table 1.
Preparation of Pyruvate Standards

Tube	Standard Premix	Ultrapure Water	Pyruvate (μM)
1	100 μL	0 μL	500
2	80 μL	20 μL	400
3	60 μL	40 μL	300
4	40 μL	60 μL	200
5	30 μL	70 μL	150
6	20 μL	80 μL	100
7	10 μL	90 μL	50
8	0 μL	100 μL	0

Reaction Mix

For each well of reaction, prepare Reaction Mix by mixing into a clean tube:

94 μL of Enzyme Mix
1 μL of Dye Reagent

Colorimetric Assay Reaction

1. Transfer 10 μL of standards and 10 μL of samples into separate wells of a clear flat bottom 96 well plate.
2. Add 90 μL of the Reaction Mix to each standard and sample well. Tap plate to mix well. Freeze unused reagents for future use.
3. Incubate 30 minutes at room temperature.
4. Measure the absorbance at 570 nm (A_{570}).
   Note: If the Sample (A_{570}) is higher than the 500 μM Standard (A_{570}), dilute sample in ultrapure water and repeat the assay. Multiply result by the dilution factor.

Fluorometric Procedure

For the fluorometric assay, the linear detection range is 0.2–50 μM pyruvate.

1. Dilute the Standards prepared in Colorimetric Procedure 10-fold in ultrapure water.
2. Transfer 10 μL of standards and 10 μL of samples into separate wells of a black 96 ell plate.
3. Add 90 μL of the Reaction Mix to each standard and sample well. Tap plate to mix well. Freeze unused reagents for future use.
4. Tap plate to mix. Incubate 30 minutes at room temperature.
5. Read fluorescence at λ_{ex} = 530 nm/λ_{em} = 585 nm.
   Note: If assays in 384 well plate are desired, use 5 μL of Standards or 5 μL of samples, and 45 μL of the Reaction Mix to each standard or sample well.

Results

Subtract the blank A_{570} (Tube 8) from the standard A_{570} values and plot the A_{570} against standard concentrations. Determine the slope using linear regression fitting. Calculation of the pyruvate concentration for the Sample in the colorimetric assay:

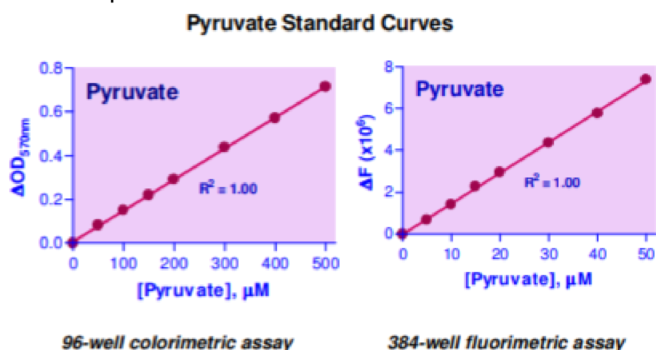
$$[\text{Pyruvate } \mu\text{M}] = \frac{A_{570\ \text{Sample}} - A_{570\ \text{Blank}}}{\text{Slope}}$$

Calculation of the pyruvate concentration for the Sample in the fluorometric assay:

$$[\text{Pyruvate } \mu\text{M}] = \frac{F_{\text{Sample}} - F_{\text{Blank}}}{\text{Slope}}$$

Figure 1.

Serum samples were run in duplicate according to the standard procedure.



References

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