Product Manual

Pyruvate Assay Kit

Catalog Number

MET-5029 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Pyruvate, the conjugate base of pyruvic acid, is an alpha-keto acid that serves as a key intermediate in several metabolic pathways. Pyruvate can be synthesized from glucose through the glycolytic pathway and converted back to carbohydrates through gluconeogenesis. Pyruvate can be converted to fatty acids through a reaction involving acetyl-CoA and can also be used in the synthesis of the amino acid alanine. Pyruvate can be converted into ethanol or lactic acid through the process of fermentation. Finally, pyruvate is a key component in providing energy to cells through the citric acid cycle under oxygen rich conditions (aerobic respiration), and can also lead to production of lactate in anaerobic environments (fermentation).

Cell Biolabs' Pyruvate Assay Kit is a simple fluorometric assay that measures the amount of total pyruvate present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, pyruvate standards, and unknown samples. Sample pyruvate concentrations are determined by comparison with a known pyruvate standard. The kit has a detection sensitivity limit of 1 µM pyruvate.

Note: Each sample replicate requires 2 assays, one treated with pyruvate oxidase (+PO) and one without (-PO). Pyruvate is calculated from the difference in OD readings from the 2 wells.

Assay Principle

Cell Biolabs' Pyruvate Assay Kit measures total pyruvate within biological samples. Pyruvate is oxidized by pyruvate oxidase in the presence of phosphate and oxygen into acetyl phosphate, carbon dioxide, and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of pyruvate standard within the 96-well microtiter plate format. Samples and standards are incubated for 30 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

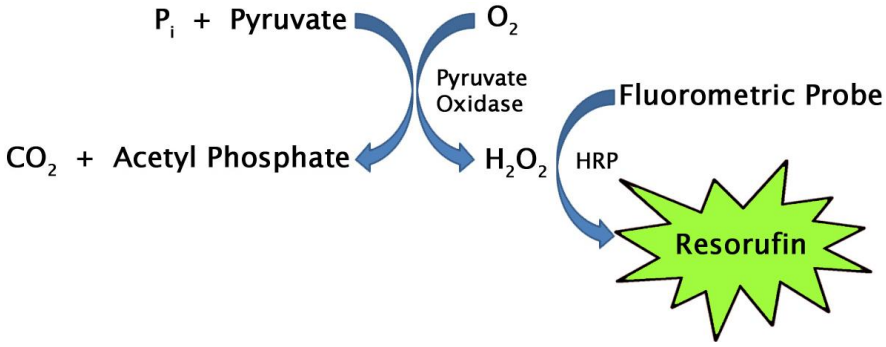

Figure 1. Pyruvate Assay Principle.

Related Products

1. STA-681: Glucose Assay Kit (Fluorometric)
2. MET-5023: Glycogen Assay Kit (Fluorometric)
3. STA-619: Free Fatty Acid Assay Kit (Fluorometric)
4. MET-5163: ATP Assay Kit (Fluorometric)

5. STA-390: Total Cholesterol Assay Kit (Fluorometric)

Kit Components (shipped on blue ice)

1. Pyruvate Standard (Part No. 50291B): One 50 µL tube at 8 mM.
2. 10X Assay Buffer (Part No. 50292A): One 25 mL bottle.
3. Fluorometric Probe (Part No. 50231C): One 50 µL tube in DMSO.
4. HRP (Part No. 234402-T): One 10 µL tube of a 100 U/mL solution in glycerol.
5. FAD (Part No. 50293C): One 50 µL tube of 2 mM Flavin Adenine Dinucleotide (FAD).
6. TPP (Part No. 50294C): One 50 µL tube of 2 mM Thiamine Pyrophosphate (TPP).
7. Pyruvate Oxidase (Part No. 50295C): One 300 µL tube.

Materials Not Supplied

1. Distilled or deionized water
2. 10 kDa molecular weight cutoff (MWCO) centrifuge spin filter (e.g. Amicon Ultra 0.5 mL)
3. Phosphate Buffered Saline (PBS)
4. Standard 96-well fluorescence black microtiter plate and/or black cell culture microplate

Storage

Upon receipt, store the Pyruvate Standard and 10X Assay Buffer at 4°C. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. Store at 4°C.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, Pyruvate Oxidase 1:16.7, FAD 1:100, and TPP 1:100 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, 60 µL of Pyruvate Oxidase, 10 µL of FAD, and 10 µL of TPP to 908 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.
- Negative Control Reaction Mix: Prepare a Negative Control Reaction Mix without Pyruvate Oxidase by diluting the Fluorometric Probe 1:100, HRP 1:500, FAD 1:100, and TPP 1:100 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP, 10 µL of FAD, and 10 µL of TPP to stock solution to 968 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell culture supernatants: Cell culture media formulated with pyruvate should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary in PBS.

Note: Maintain pH between 7 and 8 for optimal working conditions as the Fluorometric Probe is unstable at high pH (>8.5).

- Tissue lysates: Sonicate or homogenize tissue sample in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in PBS.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma, saliva, or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in PBS.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Pyruvate standards by diluting in PBS according to Table 1.

Standard Tubes	8 mM Pyruvate Solution (µL)	PBS (µL)	Pyruvate (µM)	Pyruvate (mg/dL)
1	5	495	80	0.704
2	250 of Tube #1	250	40	0.352
3	250 of Tube #2	250	20	0.176
4	250 of Tube #3	250	10	0.088
5	250 of Tube #4	250	5	0.044
6	250 of Tube #5	250	2.5	0.022
7	250 of Tube #6	250	1.25	0.011
8	0	250	0	0

Table 1. Preparation of Pyruvate Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with Pyruvate Oxidase (+PO) and one without the enzyme (-PO) to measure endogenous background (1X Assay buffer will be added to the Negative Control Reaction Mix in place of Pyruvate Oxidase).

2. Add 50 µL of each pyruvate standard or unknown sample into wells of a 96-well microtiter plate.

3. Add 50 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 50 µL of Negative Control Reaction Mix to the other half of the paired sample wells.
5. Mix the well contents thoroughly and incubate for 30 minutes at 37ºC protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
6. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Example of Results

The following figures demonstrate typical Pyruvate Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

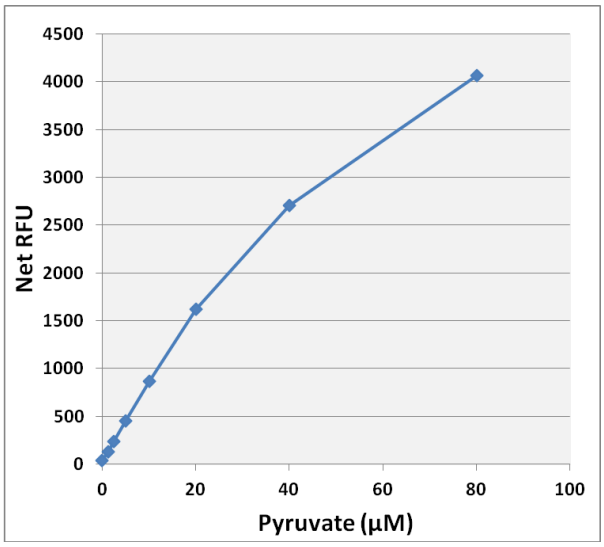

Figure 2: Pyruvate Standard Curve.

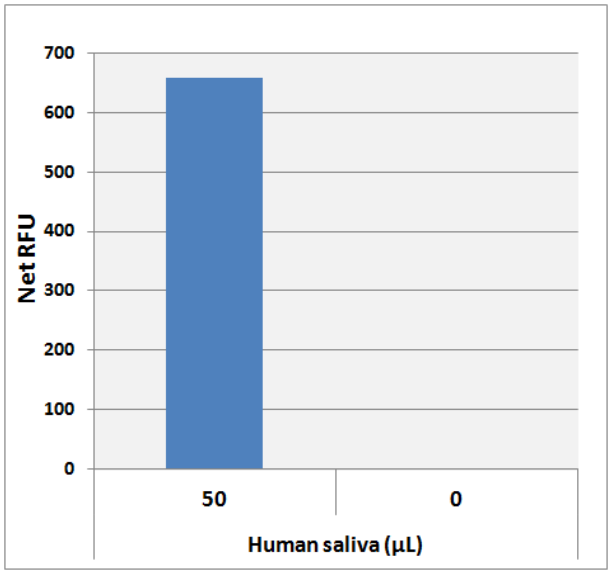

Figure 3: Pyruvate Detection in Human Saliva using the Pyruvate Assay Kit.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Pyruvate Oxidase (-PO) from the sample well values containing Pyruvate Oxidase (+PO) to obtain the difference. The fluorescence difference is due to the Pyruvate Oxidase activity.

$$\text{Net RFU} = (\text{RFU}_{+\text{PO}}) - (\text{RFU}_{-\text{PO}})$$

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of pyruvate present in the sample. Only use values within the range of the standard curve.

References

1. Dakin HD and Janney NW (1913). *J. Biol. Chem.* **15**:177.
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.
3. Smedley I and Lubrzynska E (1913) *Biochem J*. **7**:364.
4. Ringer AI (1913) *J. Biol. Chem*. **15**:145.

Recent Product Citation

Takahiro Yamanaka, T. et al. (2025). Testosterone-Induced Metabolic Changes in Seminal Vesicle Epithelium Modify Seminal Plasma Components with Potential to Improve Sperm Motility. *Elife*. doi: 10.7554/eLife.95541.4.

Warranty

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