Product Manual

Pyruvate Assay Kit (Colorimetric)

Catalog Number

MET-5125 **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. The metabolite 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. Fermentation can convert pyruvate into ethanol or lactic acid. 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). Pyruvate has been cited as a potent antioxidant, endurance and weight loss supplement, and shown to reduce cholesterol. Elevated pyruvate levels have been associated with liver diseases and genetic disorders.

Cell Biolabs' Pyruvate Assay Kit is a simple colorimetric 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 ~3 µM pyruvate.

Assay Principle

Cell Biolabs' Pyruvate Assay Kit measures total pyruvate within biological samples. Pyruvate is oxidized by a pyruvate enzyme 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 colorimetric probe. HRP 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 30-60 minutes and then read with a standard 96-well spectrophotometric plate reader (Figure 1).

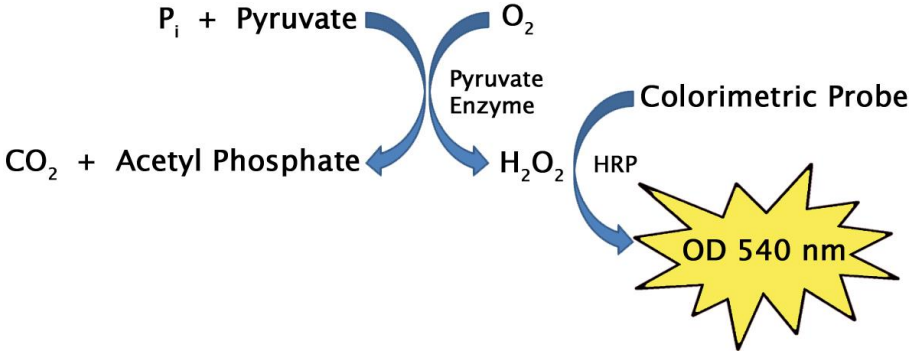

Figure 1. Pyruvate Assay Principle.

Related Products

1. MET-5001: Lactose Assay Kit
2. MET-5012: Lactate Assay Kit (Colorimetric)
3. MET-5022: Glycogen Assay Kit (Colorimetric)
4. STA-674: Glutamate Assay Kit
5. STA-680: Glucose Assay Kit (Colorimetric)

Kit Components

1. Pyruvate Enzyme Mix (Part No. 51251C): One 900 µL amber tube
2. Cofactor (Part No. 51252C): One 200 µL amber tube
3. Pyruvate Standard (Part No. 51243C): One 100 µL tube of a 10 mM solution
4. Colorimetric Probe (Part No. 51253C): One 150 µL tube
5. HRP (Part No. 234402): One 100 µL tube of a 100 U/mL solution in glycerol
6. Assay Buffer 10X (Part No. 50292A): One 25 mL bottle

Materials Not Supplied

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

Storage

Upon receipt, store the 10X Assay Buffer at 4°C. Store all other components at -20ºC. The Colorimetric 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 Mixture by diluting the Colorimetric Probe 1:100, HRP 1:500, Pyruvate Enzyme Mix 1:17, and Cofactor 1:100 in 1X Assay Buffer. See Table 1 below for examples of Reaction Mix preparation based on the number of assays employed. Mix thoroughly and protect the solution from light. For best results, place the Reaction Mix on ice and use within 30 minutes of preparation. Do not store the Reaction Mix solution.

Pyruvate Enzyme Mix (µL)	Cofactor (µL)	HRP (µL)	Colorimetric Probe (µL)	1X Assay Buffer (mL)	Number of Assays (150 µL/well)
883	150	30	150	13.8	**100**
442	75	15	75	6.90	**50**
221	38	8	38	3.45	**25**

Table 1. Preparation of Reaction Reagent

Note: Prepare only enough for immediate use by scaling the above example proportionally. The Colorimetric Probe is light sensitive and must be stored accordingly.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. It is highly recommended that all samples should be assayed immediately upon preparation or stored for up to 1 month at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results.

Samples may be deproteinized with a 10kDa MWCO spin filter prior to the assay. Run proper controls and account for any sample dilutions. Always run a standard curve with samples.

- Tissues: Weigh 500-1000 mg of sample and mince with scissors and a dounce until tissue is thoroughly liquified. Add 2 mL of 1X Assay Buffer or PBS and further sonicate the homogenate for several cycles on ice. Centrifuge 10 minutes at 12,000 x g to remove debris. Recover the supernatant and recentrifuge in a separate tube to clarify it further. Recover supernatant in a fresh eppendorf tube and incubate on ice. Prepare samples for testing and store the remaining supernatant at -80ºC. Prepare further dilutions in 1X Assay Buffer or PBS.
- Cell Lysate: 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: Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform serum dilutions in 1X Assay Buffer or PBS. Perform several serial dilutions to ensure values are within the range of the standard curve.
- Plasma: Collect blood with heparin or citrate (EDTA might cause a quenching effect) and centrifuge at 1000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Perform plasma dilutions in 1X Assay Buffer or PBS. Perform several serial dilutions to ensure values are within the range of the standard curve.
- 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 1X Assay Buffer or PBS.
- 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 1X Assay Buffer or 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.*
- *Lactate Dehydrogenase (LDH), as well as other enzymes, can react with pyruvate or reaction constituents, and convert pyruvate to lactate. If LDH is present, samples may need to be deproteinated prior to testing.*
- *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 Colorimetric Probe is not stable in the presence of thiols (above 10 µM).*
- *The Colorimetric Probe is unstable at high pH (>8.5).*

Preparation of Standard Curve

Prepare fresh Pyruvate standards by diluting the 10 mM stock Pyruvate Standard in PBS according to Table 2. Prepare standards immediately before use and do not store standard solutions.

Standard Tubes	10 mM Pyruvate Solution (µL)	PBS (µL)	Pyruvate (µM)	Pyruvate (nmoles/well)	Pyruvate (mg/dL)
1	20	480	400	20	3.52
2	250 of Tube #1	250	200	10	1.76
3	250 of Tube #2	250	100	5	0.881
4	250 of Tube #3	250	50	2.5	0.440
5	250 of Tube #4	250	25	1.25	0.220
6	250 of Tube #5	250	12.5	0.625	0.110
7	250 of Tube #6	250	6.25	0.313	0.055
8	250 of Tube #7	250	3.13	0.156	0.028
9	0	250	0	0	0

Table 2. Preparation of Pyruvate Standards. The Molecular Weight of Pyruvate is 88.06 g/mol.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each pyruvate standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 150 µL of Reaction Mix to the standard and sample wells, and mix the well contents thoroughly.
4. Incubate the wells for 30-60 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.

5. Read the plate with a spectrophotometric microplate reader at 540-570 nm.

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.

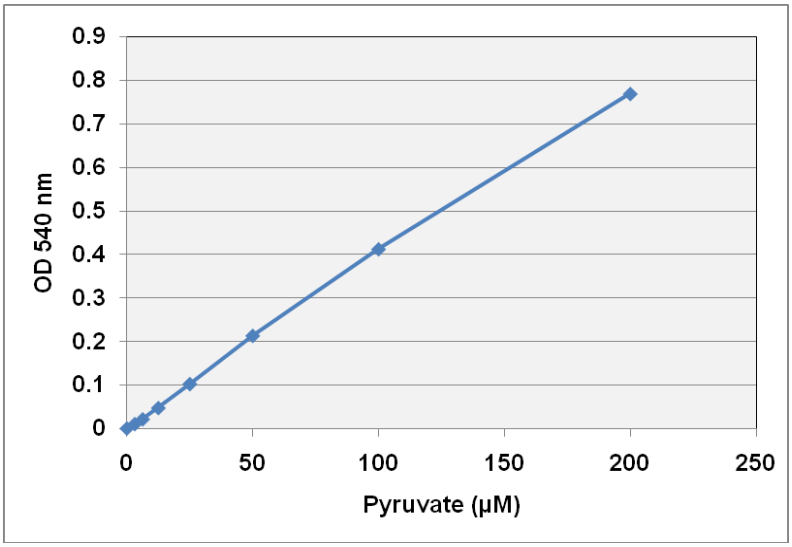

Figure 2: Pyruvate Standard Curve.

Calculation of Results

1. Calculate the average absorbance values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected absorbance.
2. Plot the corrected absorbance for the standards against the final concentration of the pyruvate standards from Table 2 to determine the best curve. Use the 60-minute reading values, or final time point absorbance values, to plot the pyruvate standard curve. See Figure 2 for an example standard curve.
3. Determine the pyruvate concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected absorbance values for each sample. Only use values within the range of the standard curve. Remember to account for dilution factors.

$$PYRUVATE\ (\mu M) = \left(\frac{Sample\ corrected\ absorbance}{Slope}\right) \times Sample\ Dilution$$

Note: For the conversion of results from µM to mg/dl, divide the pyruvate concentration (µM) by 113.56.

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

Amrutkar, M. et al. (2022). Neoadjuvant chemotherapy is associated with an altered metabolic profile and increased cancer stemness in patients with pancreatic ductal adenocarcinoma. *Mol Oncol*. doi: 10.1002/1878-0261.13344.

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