MANUAL

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Pyruvate Assay Kit (Colorimetric)

Catalog #: MA-PYRU

ISO 13485:2016

Introduction

Pyruvate, the conjugate base of pyruvic acid, is an alpha-keto acid that serves as a key intermediate in several metabolic pathways. It is synthesized from glucose through the glycolytic pathway and can be converted back to carbohydrates through gluconeogenesis. Pyruvate plays a role in the synthesis of fatty acids via a reaction involving acetyl-CoA and contributes to the production of the amino acid alanine. Pyruvate serves as a vital component in providing cells energy through the citric acid cycle during aerobic respiration in oxygen-rich conditions and leads to the production of lactate in anaerobic environments through fermentation. Pyruvate has been recognized for its antioxidant properties and has been investigated for its potential benefits in enhancing endurance, aiding weight loss, and reducing cholesterol levels. Elevated levels of pyruvate have been associated with liver diseases and genetic disorders.

Pyruvate Assay Kit provides a simple, reproducible, and sensitive tool of measuring pyruvate concentration in plasma, serum, cell lysates, saliva, urine, and other biological liquid samples. This assay employs a coupled enzymatic reaction system that combines multiple steps. Initially, pyruvate oxidase catalyzes the conversion of pyruvate to acetyl-phosphate, hydrogen peroxide (H_2O_2), and carbon dioxide. Peroxidase then catalyzes the reaction of hydrogen peroxide with the Colorimetric Probe to form a pink colored product. The optical density measured at 520nm is directly proportional to the concentration of pyruvate present in the sample.

Storage

The entire kit should be stored below -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	25 ml	2–8 °C, 3 months
Pyruvate Standard	1 vial (20 µl of 100mM)	2–8 °C, 1 week
Enzyme Mix	2 amber vials (Lyophilized)	Do not store and reuse.
Cofactor	2 amber vials (Lyophilized)	Do not store and reuse.
Colorimetric Probe	2 amber vials (Lyophilized)	Do not store and reuse.

RT = room temperature
**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 520 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Tubes to prepare sample dilutions
4. Incubator at 37°C
5. 50 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, normal human plasma has pyruvate concentrations in the range of 60-150 µM. The sample may be assayed directly or diluted as necessary in Assay Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, normal human serum has pyruvate concentrations in the range of 60-150 µM. The sample may be assayed directly or diluted as necessary in Assay Buffer.

4. Saliva, or Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. The supernatant may be assayed directly or diluted as necessary in Assay Buffer.

Reagent Preparation

REAGENT	PREPARATION
Microplate	No preparation
Assay Buffer	
Pyruvate Standard	See “Standard Preparation” section
Enzyme Mix	For each vial, reconstitute with 1 ml Assay Buffer.
Cofactor	For each vial, reconstitute with 1 ml Assay Buffer.
Colorimetric Probe	For each vial, reconstitute with 1 ml Assay Buffer.
Working Solution	Add 1 mL reconstituted Enzyme Mix, 1 mL reconstituted Cofactor, and 1 mL reconstituted Colorimetric Probe into 5 mL Assay Buffer to make the **Working Solution**. This is enough to assay 60 wells. Prepare the additional vial as needed. The Working Solution is stable for 1 day at 2-8°C in the dark.

Standard Preparation

To prepare a dilution series of Pyruvate Standard in the concentration range of 0 µM – 400 µM (see Table below),

1. Label 8 microtubes #1 through 8 with the following concentrations: 400, 200, 100, 50, 25, 12.5, 6.25, 0 µM.
2. Pipette 498 µL Assay Buffer into labeled tube #1, and 250 µL Assay Buffer into labeled tube #2 through #8.
3. Pipette 2 µL of Pyruvate Standard into tube#1, mix thoroughly and quick spin, to make a 400 µM pyruvate Standard stock.
4. To make the 200 µM standard, pipette 250 µL of tube #1 into the tube labeled #2. Mix thoroughly and quick spin.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 250 µl of the prior concentration until the 6.25 µM is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh and used immediately.

Labeled Tubes	Pyruvate Standard (µL)	Assay Buffer (µL)	Standard Conc. (µM)
1	2 µL of pyruvate Standard	498 µL	400
2	250 µL of Tube #1	250 µL	200
3	250 µL of Tube #2	250 µL	100
4	250 µL of Tube #3	250 µL	50
5	250 µL of Tube #4	250 µL	25
6	250 µL of Tube #5	250 µL	12.5
7	250 µL of Tube #6	250 µL	6.25
8	0µL	250 µL	0

Assay Procedure

Each Pyruvate Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 50 µL of the diluted Pyruvate Standards or samples to the 96-well microtiter plate.
2. Initiate the reaction by adding 150 µL Working Solution (See “Reagent Preparation”) to each well. Carefully shake the plate for a few seconds to mix.
3. Incubate the plate for 30 minutes at 37ºC with mixing in the dark.
4. Measure the absorbance at 520nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 µM) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 520nm as a function of pyruvate concentration.

Determination of sample pyruvate concentration

Pyruvate (µM) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

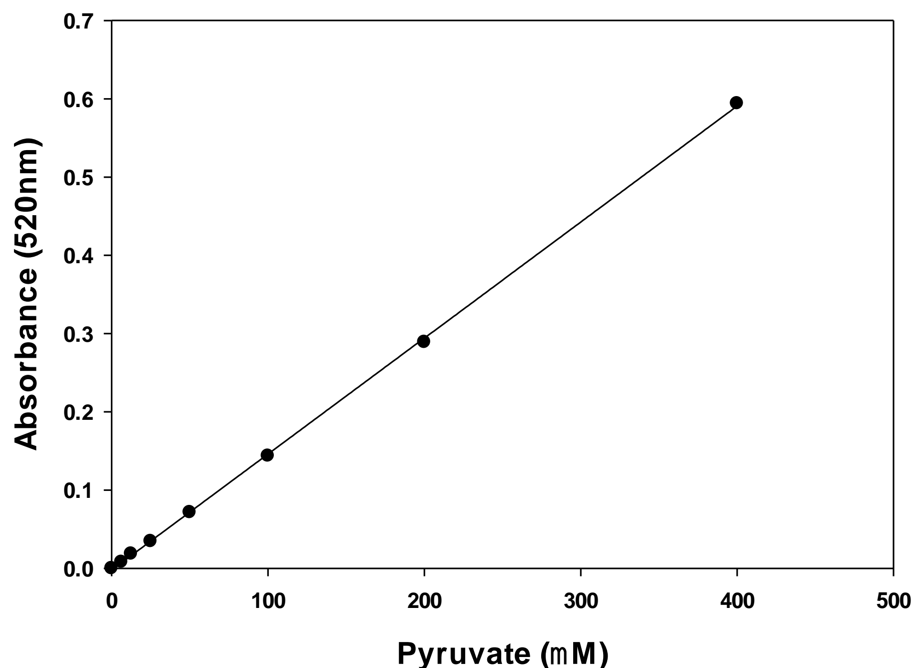
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 µM)

Slope is from the plot of pyruvate concentration vs. Absorbance (as shown in Typical data below).

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated pyruvate concentration of the sample is higher than 400 µM, dilute the sample in assay buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Sensitivity

The minimum detectable concentration of pyruvate is 3 µM.
Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.8%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 3.2%.

This product is for research use only.