Pyruvate Assay Kit

Item No. 700470

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, store the kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700471	Pyruvate Assay Buffer (10X)	1 vial/10 ml	-20°C
700472	Pyruvate Assay Cofactor Mixture	2 vials	-20°C
700473	Pyruvate Assay Enzyme Mixture	2 vials	-20°C
700474	Pyruvate Assay Fluorometric Detector	3 vials	-20°C
700475	Pyruvate Assay Standard	2 vials	-20°C
700001	DMSO Assay Reagent	1 vial/1 ml	RT
700518	MPA Assay Reagent	1 vial/2 g	RT
700517	Potassium Carbonate Assay Reagent	1 vial/5 ml	-20°C
400017	96-Well Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

It is recommended to take appropriate precautions when using the kit reagents (*i.e.*, lab coat, gloves, eye goggles, etc.) as some of them may be harmful. MPA (metaphosphoric acid) and potassium carbonate are corrosive and harmful if swallowed. Contact with skin may cause burns. In case of contact with skin or eyes, rinse immediately with plenty of water for 15 minutes.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Pyruvate (pyruvic acid) is a key intermediate in cellular metabolic pathways. Pyruvate is primarily derived from glucose *via* glycolysis. Provided that sufficient oxygen is available, pyruvate is converted by pyruvate dehydrogenase into acetyl-CoA which enters the citric acid cycle (Kreb's cycle) where it is metabolized to produce ATP. Pyruvate can also be converted into carbohydrates *via* gluconeogenesis or to fatty acids through acetyl-CoA.

Recently, pyruvate has been used as a dietary supplement to aid in weight loss.[1] Pyruvate can improve exercise endurance capacity, effectively reduce cholesterol, and serves as a potent antioxidant.[2] Therefore, the measurement of its concentration can give valuable information to the progress of specific biochemical reactions.

The lactate to pyruvate ratio reflects the redox state of the cell and describes the balance between NAD^+ and NADH, which is dependent on the interconversion of lactate and pyruvate *via* lactate dehydrogenase. This ratio has been used to assess the severity of circulatory failure as well as other disease states. Abnormal blood pyruvate levels are reported in a number of disorders including shock, liver disease, congestive heart failure, diabetes mellitus, thiamine deficiency, and metabolic disorders.[3-6]

About This Assay

Cayman's Pyruvate Assay provides a fluorescence-based method for quantifying pyruvate in biological samples such as serum, plasma, blood, urine, and saliva. It can also be utilized to determine intracellular and extracellular pyruvate concentrations in cell culture samples. In the assay, pyruvate oxidase catalyzes the conversion of pyruvate to acetyl phosphate, hydrogen peroxide (H_2O_2), and carbon dioxide. In the presence of horseradish peroxidase, H_2O_2 reacts stoichiometrically with 10-acetyl-3,7,-dihydroxyphenoxazine (ADHP) to produce the highly fluorescent compound resorufin (Figure 1). Resorufin fluorescence is analyzed with an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm.

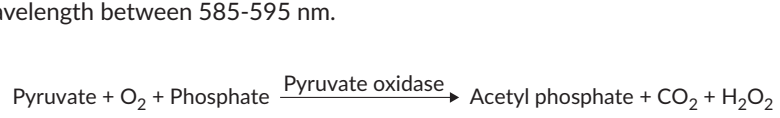


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Pyruvate Assay Buffer (10X) - (Item No. 700471)**

   The vial contains 10 ml of 1 M potassium phosphate, pH 7.0, containing 10 mM EDTA and 10 mM $MgCl_2$. Dilute 5 ml of Assay Buffer concentrate with 45 ml of HPLC-grade water. The diluted Assay Buffer (100 mM potassium phosphate, pH 7.0, containing 1 mM EDTA and 1 mM $MgCl_2$) is used in the assay. The diluted buffer is stable for six months at 4°C.

2. **Pyruvate Assay Cofactor Mixture - (Item No. 700472)**

   The vial contains a lyophilized powder of FAD and thiamine pyrophosphate. Reconstitute the contents of the vial with 3 ml of diluted Assay Buffer. This is enough cofactor mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted mixture is stable for two weeks at -20°C.

3. **Pyruvate Assay Enzyme Mixture - (Item No. 700473)**

   The vial contains a lyophilized powder of pyruvate oxidase and horseradish peroxidase. Reconstitute the contents of the vial with 1.2 ml of diluted Assay Buffer and place on ice. This is enough enzyme mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted enzymes are stable for two weeks at -20°C.

4. **Pyruvate Assay Fluorometric Detector - (Item No. 700474)**

   The vial contains a clear lyophilized powder of 10-acetyl-3,7-dihydroxyphenoxazine (ADHP). Immediately prior to use, add 100 µl of DMSO Assay Reagent (Item No. 700001) to the vial and vortex. Then add 1.1 ml of diluted Assay Buffer and vortex. This is enough detector to assay 96 wells. The reconstituted mixture is stable for 60 minutes. After 60 minutes, increased background fluorescence will occur.

5. **Pyruvate Assay Standard - (Item No. 700475)**

   The vial contains a lyophilized powder of pyruvate. Reconstitute the contents of the vial with 1 ml of diluted Assay Buffer to prepare a 10 mM pyruvate stock. It is now ready to use to prepare the standard curve.

6. **DMSO Assay Reagent - (Item No. 700001)**

   The vial contains 1 ml of dimethylsulfoxide (DMSO). The reagent is ready to use as supplied.

7. **MPA Assay Reagent - (Item No. 700518)**

   The vial contains 2 g of metaphosphoric acid (MPA). To prepare 0.5 M MPA for deproteinating the samples, dissolve 1.6 g of MPA in 40 ml of HPLC-grade water. Store the diluted acid solution at room temperature. The diluted acid is stable for three months at room temperature.

8. **Potassium Carbonate Assay Reagent - (Item No. 700517)**

   The vial contains 5 ml of 5 M potassium carbonate. The reagent is ready to use as supplied.

Sample Preparation

Due to the presence of lactate dehydrogenase in samples, care must be taken during sample processing to prevent the conversion of pyruvate to lactate. It is important for samples to be deproteinated upon collection and then they can be stored at -80°C.[7]

Plasma

Typically, normal human plasma has a pyruvate concentration in the range of 60-150 µM.

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 25°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer.
3. To deproteinate 500 µl of plasma, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
4. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
5. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
6. If not assaying the same day, freeze at -80°C. The deproteinated plasma sample will be stable for one month while stored at -80°C.
7. Dilute the plasma sample 1:2 with diluted Assay Buffer before assaying.

Serum

Typically, normal human serum has a pyruvate concentration in the range of 60-150 µM.

1. Collect blood without using an anticoagulant.
2. Allow the blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 25°C. Pipette off the top yellow serum layer without disturbing the white buffy layer.
4. To deproteinate 500 µl of serum, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
5. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
6. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
7. If not assaying the same day, freeze at -80°C. The deproteinated serum sample will be stable for one month while stored at -80°C.
8. Dilute the serum sample 1:2 with diluted Assay Buffer before assaying.

Blood

Typically, normal human blood has a pyruvate concentration in the range of 35-100 µM.

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. To deproteinate 500 µl of blood, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated blood sample will be stable for one month while stored at -80°C.
6. Dilute the blood sample 1:2 with diluted Assay Buffer before assaying.

Saliva

Typically, normal human saliva has a pyruvate concentration in the range of 18-130 µM.

1. Collect saliva in a clean test tube.
2. To deproteinate 500 µl of saliva, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated saliva sample will be stable for one month while stored at -80°C.
6. Saliva does not need to be diluted before assaying.

Urine

1. Collect urine in a clean container.
2. To deproteinate 500 µl of urine, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
3. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
4. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
5. If not assaying the same day, freeze at -80°C. The deproteinated urine sample will be stable for one month while stored at -80°C.
6. Urine does not need to be diluted before assaying.

NOTE: Pyruvate values from urine samples can be standardized using Cayman's Creatinine Assay Kit (Item No. 500701).

Extracellular Pyruvate

1. Remove sufficient cell culture medium (~10-12 ml) from a culture flask to obtain ≥22 x 10^6 cells (cell density ~2 x 10^6 cells/ml) and centrifuge at 1,000 x g for five minutes.
2. The cell pellet is used for intracellular pyruvate determination (see below).
3. To deproteinate 500 µl of supernatant, add 500 µl of cold 0.5 M MPA, vortex, and place on ice for five minutes.
4. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 50 µl of Potassium Carbonate to neutralize the acid.
5. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
6. If not assaying the same day, freeze at -80°C. The deproteinated sample will be stable for one month while stored at -80°C.
7. Dilute the extracellular sample 1:2 with diluted Assay Buffer before assaying.

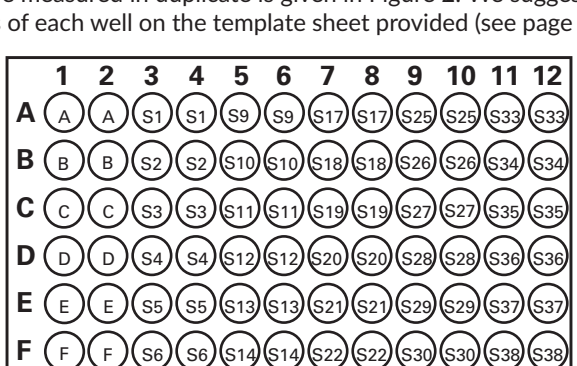
Intracellular Pyruvate

1. Add 1 ml of PBS to the cell pellet to wash the cells. A cell count can be performed at this time.
2. Centrifuge at 10,000 x g for five minutes at 4°C. Discard the supernatant.
3. To prepare 0.25 M MPA, dilute 1 ml of 0.5 M MPA with 1 ml of HPLC-grade water.
4. To deproteinate the sample, add 0.5 ml of 0.25 M MPA to the cell pellet, vortex, and place on ice for five minutes.
5. Centrifuge at 10,000 x g for five minutes at 4°C to pellet the proteins. Remove the supernatant and add 25 µl of Potassium Carbonate to neutralize the acid.
6. Centrifuge at 10,000 x g for five minutes at 4°C to remove any precipitated salts. Remove the supernatant for assaying.
7. If not assaying the same day, freeze at -80°C. The deproteinated sample will be stable for one month while stored at -80°C.
8. Dilute the intracellular sample 1:2 with diluted Assay Buffer before assaying.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a pyruvate standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate. A typical layout of standards and samples to be measured in duplicate is given in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 26).



A-H = Standards
S1-S40 = Sample wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 150 µl in all the wells.
- All reagents except the enzymes must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples at least in duplicate (triplicate preferred), but it is the user's discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm.

Standard Preparation

Take eight clean glass test tubes and mark them A-H. To prepare the 1 mM pyruvate stock, dilute 100 µl of 10 mM Pyruvate with 900 µl of diluted Assay Buffer. Add the amount of pyruvate stock (1 mM) and Assay Buffer to each tube as described in Table 1. The diluted standards are stable for four hours at room temperature.

Tube	1 mM Pyruvate stock (µl)	Assay Buffer (µl)	Final Concentration (µM)
A	0	1,000	0
B	3	997	3
C	6	994	6
D	15	985	15
E	30	970	30
F	45	955	45
G	60	940	60
H	75	925	75

Table 1. Preparation of pyruvate standards

Performing the Assay

1. **Standard Wells** - add 50 µl of Assay Buffer, 50 µl of Cofactor Mixture, 10 µl Fluorometric Detector, and 20 µl of Standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 2, page 16).
2. **Sample Wells** - add 50 µl of Assay Buffer, 50 µl of Cofactor Mixture, 10 µl of Fluorometric Detector, and 20 µl of sample to at least two wells.
3. Initiate the reactions by adding 20 µl of Enzyme Mixture to all of the wells being used.
4. Incubate the plate for 20 minutes at room temperature and then read using an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm. Fluorescence is stable up to 45 minutes.

ANALYSIS

Calculations

1. Determine the average fluorescence of each standard and sample.
2. Subtract the fluorescence value of the standard A from itself and all other standards and samples. This is the corrected fluorescence (CF).
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final concentration of pyruvate from Table 1. See Figure 3, on page 21, for a typical standard curve.
4. Calculate the pyruvate concentration of the samples using the equation below.

$$\text{Pyruvate (µM)} = \left[\frac{\text{(CF - y-intercept)}}{\text{Slope}}\right] \times 2^{*} \times \text{Sample dilution}$$

*This is a dilution factor to correct for deproteinating the samples with 0.5 M MPA. Do not use this dilution factor when determining intracellular pyruvate values.

NOTE: Pyruvate values from urine samples can be standardized using Cayman's Creatinine Assay Kit (Item No. 500701).

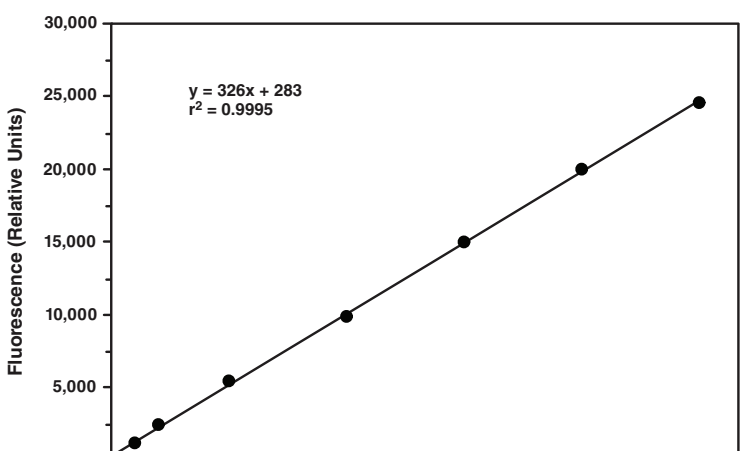


Figure 3. Pyruvate standard curve

Performance Characteristics

Precision:

When a series of eight deproteinated blood or eight deproteinated plasma mesurements were performed on the same day, the intra-assay coefficient of variation was 1.2% and 2.4%, respectively. When a series of eight deproteinated blood or eight deproteinated plasma measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 2.0% and 2.8%, respectively.

Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-75 µM pyruvate.

Assay Specificity:

To assess substrate specificity, the assay was performed with pyruvate replaced by structurally similar compounds. Compounds such as lactate and α-keto acids might interfere with the measurement of pyruvate, so fluorescence signals generated by pyruvate and other α-keto acids as substrates under identical conditions were evaluated. Lactate and phosphoenolpyruvate (PEP) were not utilized by pyruvate oxidase. 2-Oxobutyrate and oxaloacetate had ~8% and 3% conversion, respectively. See Figure 4, on page 23.

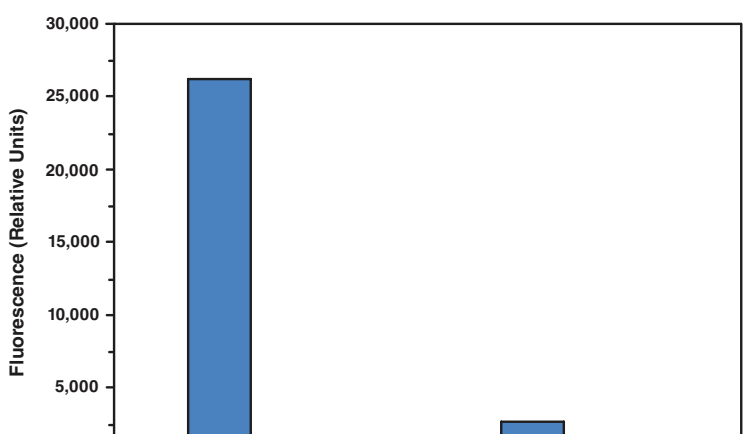


Figure 4. Assay specificity

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No fluorescence detected above background in the sample wells	Sample was too dilute	Re-assay the sample using a lower dilution
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
The fluorescence of the sample wells were higher than the last standard	Sample was too concentrated	Re-assay the sample using a higher dilution

References

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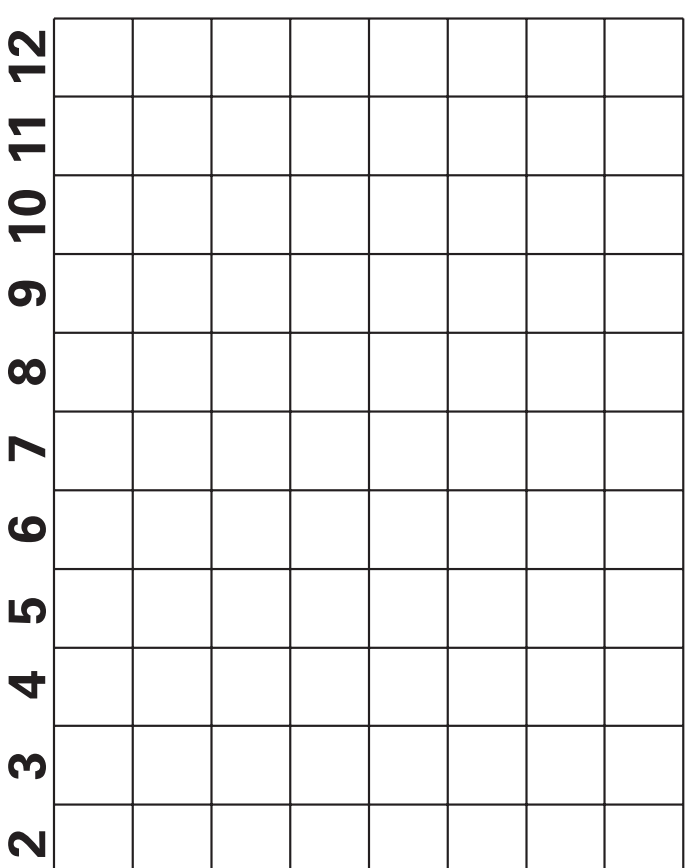

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.