Pyruvate Assay Kit

Item No. 700470

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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 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. 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

Email: techserv@caymanchem.com

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 or a plate reader capable of reading absorbance at 570 nm.
2. Adjustable pipettes and a multichannel or repeating pipettor
3. A source of pure water; glass-distilled water or HPLC-grade water is acceptable
4. A clear-bottom polystyrene plate if absorbance will be measured

INTRODUCTION

Background

Pyruvate is a key intermediate in cellular metabolism.[1] It is formed primarily from glucose *via* glycolysis but can also be derived from certain amino acids and lactate. Pyruvate is converted by the pyruvate dehydrogenase complex into acetyl CoA, which enters the citric acid cycle to produce ATP. The NADH formed by this conversion is used for mitochondrial electron transport and β-oxidation. Pyruvate can also be converted back into glucose *via* gluconeogenesis when sufficient oxygen is present. When oxygen is not present, pyruvate is converted into lactate.

Pyruvate accumulates in the blood and urine of individuals with pyruvate dehydrogenase deficiency, an inborn error of metabolism characterized by mutations in the genes encoding pyruvate dehydrogenase complex enzymes, that leads to mild-to-severe metabolic or neurological impairments.[2] Defects in pyruvate metabolism also play a role in several disease states, including cancer, neurodegenerative diseases, and heart failure.[3]

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.[4] Defects in pyruvate metabolism can be identified by an increase in blood lactate in conjunction with a normal lactate-to-pyruvate ratio, and defects in mitochondrial electron transport are evidenced by an increased lactate-to-pyruvate threshold.

About This Assay

Cayman's Pyruvate Assay provides both a fluorescence- and absorbance-based methods 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 (HRP), H_2O_2 reacts stoichiometrically with 10-acetyl-3,7,-dihydroxyphenoxazine (ADHP) to produce the compound resorufin (Figure 1). Resorufin fluorescence is analyzed with an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm. Resorufin absorbance is measured at a wavelength of 570 nm.

This assay offers the option to measure absorbance or fluorescence. It is at the user's discretion to choose the mode of measurement (and corresponding standard curve preparation) that best fits their needs. When read fluorometrically, this assay has a range of 0-75 µM and a lower limit of quantification (LLOQ) of 1.5 µM. When read colorimetrically, the range is 0-150 µM with an LLOQ of 3.0 µM.

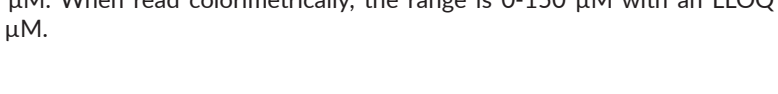


Figure 1. Assay scheme

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 (10X) with 45 ml of pure water. This Assay Buffer (1X) (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 Assay Buffer (1X). 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 HRP. Reconstitute the contents of the vial with 1.2 ml of Assay Buffer (1X) 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 Detector - (Item No. 700474)**

   The vial contains a clear lyophilized powder of ADHP. Immediately prior to use, add 100 µl of DMSO Assay Reagent to the vial and vortex. Then add 1.1 ml of Assay Buffer (1X) and vortex. This is enough Detector to assay 96 wells. The reconstituted mixture is stable for 60 minutes. After 60 minutes, increased background fluorescence or absorbance 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 Assay Buffer (1X) to prepare a 10 mM pyruvate stock solution. Prior to the start of the assay, prepare a 1 mM Pyruvate Assay Standard solution by adding 100 µl of the 10 mM pyruvate stock solution to 900 µl of Assay Buffer (1X).

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

   The vial contains 1 ml of DMSO, which is ready to use as supplied.

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

   The vial contains 2 g of metaphosphoric acid (MPA). Dissolve 1.6 g of MPA in 40 ml of pure water to prepare a 0.5 M MPA stock solution for deproteinating the samples. 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, which 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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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 Assay Buffer (1X) before assaying.

NOTE: Plasma may also be deproteinated using ultrafiltration. Plasma should be filtered using 10 kDa spin filters, following the manufactor's protocol.

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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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 Assay Buffer (1X) before assaying.

NOTE: Serum may also be deproteinated using ultrafiltration. Serum should be filtered using 10 kDa spin filters, following the manufactor's protocol.

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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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 Assay Buffer (1X) before assaying.

NOTE: Blood may also be deproteinated using ultrafiltration. Blood should be filtered using 10 kDa spin filters, following the manufactor's protocol.

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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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.

NOTE: Saliva may also be deproteinated using ultrafiltration. Saliva should be filtered using 10 kDa spin filters, following the manufactor's protocol.

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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium carbonate to it to neutralize the acid. The protein pellet may be discarded.
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.

It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

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 page 16).
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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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 Assay Buffer (1X) 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 pure 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. Transfer the supernatant into a clean test tube and add 50 µl of Potassium Carbonate to it to neutralize the acid. The protein pellet may be discarded.
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 Assay Buffer (1X) 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 must 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 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standards
S1-S40 = Sample wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette 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 at 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 or monitor the absorbance at 570 nm.

NOTE: This assay can be read using fluorescence or absorbance. You do not need to prepare both standard curves; choose the one that matches the format you will be using.

Standard Curve Preparation-Fluorometric

Label eight clean glass test tubes A-H. Add the amount of 1 mM pyruvate solution and Assay Buffer (1X) to each tube as described in Table 1, below, for the fluorometric method. The diluted standards will be stable for four hours at room temperature.

Tube	1 mM Pyruvate Solution (µl)	Assay Buffer (1X) (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 for fluorometric measurement

Standard Curve Preparation-Colorimetric

Label eight clean glass test tubes A-H. Add the amount of 1 mM pyruvate solution and Assay Buffer (1X) to each tube as described in Table 2, below, for the colorimetric method. The diluted standards will be stable for four hours at room temperature.

Tube	1 mM Pyruvate Solution (µl)	Assay Buffer (1X) (µl)	Final Concentration (µM)
A	0	1,000	0
B	6	994	6
C	12	988	12
D	30	970	30
E	60	940	60
F	90	910	90
G	120	880	120
H	150	850	150

Table 2. Preparation of pyruvate standards for colorimetric measurement

Performing the Assay

1. **Standard Wells -** add 50 µl of the diluted Assay Buffer, 50 µl of Cofactor Mixture, 10 µl of 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 17).
2. **Sample Wells -** add 50 µl of the diluted Assay Buffer, 50 µl of Cofactor Mixture, 10 µl of 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. Cover the plate with 96-Well Cover Sheet (Item No. 400012) and incubate the plate for 20 minutes at room temperature. Remove cover sheet and read fluorescence with an excitation wavelength between 530-540 nm and emission wavelength between 585-595 nm or read the absorbance at 570 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence or absorbance of each standard and sample.
2. Subtract the fluorescence or absorbance value of the standard A from itself and all other standards and samples. This is the corrected signal (CS).
3. Plot the corrected values (from step 2 above) of each standard as a function of the final concentration of pyruvate from Table 1 or 2 for the fluorometric or colorimetric methods, respectively. See Figures 3 and 4, on pages 23 and 24, respectively, for typical standard curves.
4. Calculate the pyruvate concentration of the samples using the equation below.

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

*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.

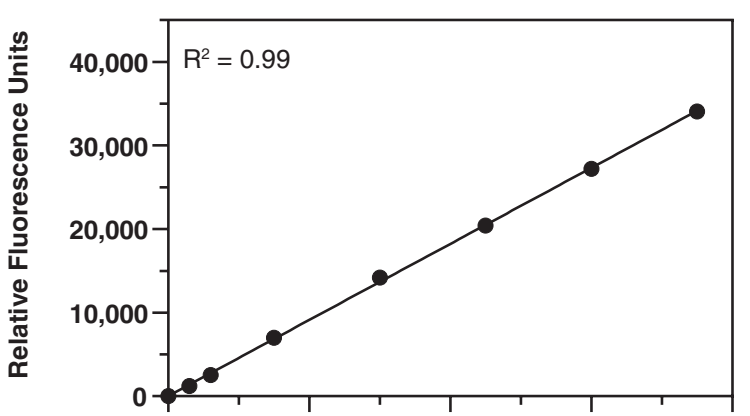


Figure 3. Pyruvate standard curve - fluorometric

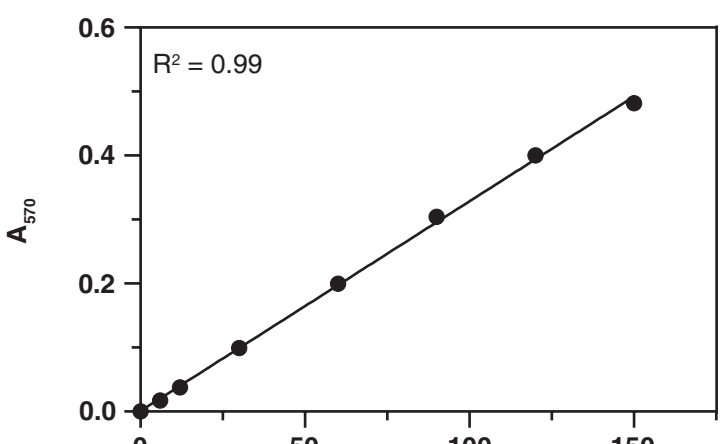


Figure 4. Pyruvate standard curve - colorimetric

Performance Characteristics

Precision:

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

Sensitivity:

The Lower Limit of Quantification (LLOQ) for the fluorometric assay is 1.5 µM. The LLOQ for the colorimetric assay is 3.0 µM.

The Lower Limit of Detection (LLOD) for both assays is 0.75 µM.

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 5, on page 26.

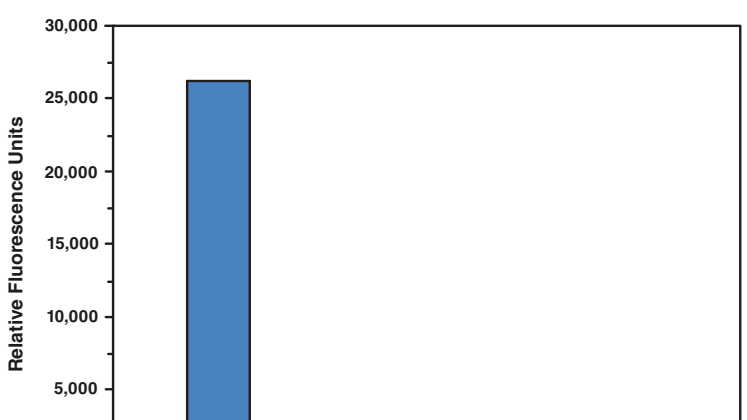


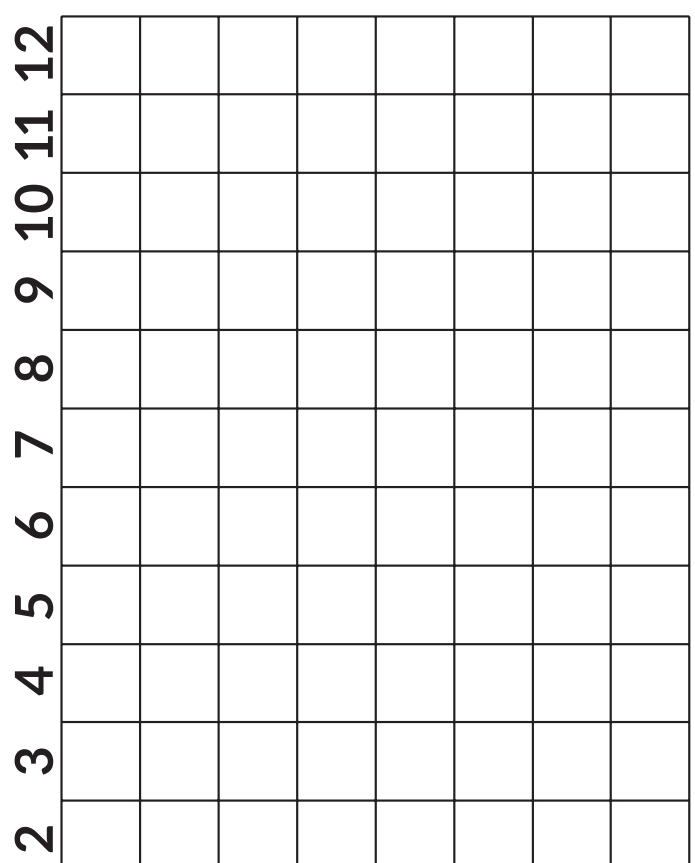
Figure 5. Assay specificity

RESOURCES

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 signal 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 signal of the sample wells were higher than the last standard	Sample was too concentrated	Re-assay the sample using a higher dilution

References

1. Voet, D. and Voet, J.G. *Biochemistry* (2004).
2. Ganetzky, R., McCormick, E.M., and Falk, M.J. Primary pyruvate dehydrogenase complex deficiency overview. In *GeneReviews*® (2021).
3. Gray, L.R., Tompkins, S.C., and Taylor, E.B. Regulation of pyruvate metabolism and human disease. *Cell. Mol. Life Sci.* **71(14)**, 2577-2604 (2014).
4. Schillaci, L.-A.P., DeBrosse, S.D., and McCandless, S.E. Inborn errors of metabolism with acidosis: Organic acidemias and defects of pyruvate and ketone body metabolism. *Pediatr. Clin. North Am.* **65(2)**, 209-230 (2018).



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.