Pyruvate Assay kit

ARG83366 Pyruvate Assay kit is an assay kit for Pyruvate in Serum, plasma, saliva, urine, Cell culture supernatants, cell lysate and tissue lysates.

Catalog number: ARG83366

Package: 100 assay

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INTRODUCTION

Pyruvate is an important chemical compound in biochemistry. It is the output of the metabolism of glucose known as glycolysis. One molecule of glucose breaks down into two molecules of pyruvate, which are then used to provide further energy, in one of two ways. Pyruvate is converted into acetyl-coenzyme A, which is the main input for a series of reactions known as the Krebs cycle (also known as the citric acid cycle or tricarboxylic acid cycle). Pyruvate is also converted to oxaloacetate by an anaplerotic reaction, which replenishes Krebs cycle intermediates.

PRINCIPLE OF THE ASSAY

ARG83366 Pyruvate Assay Kit measures total Pyruvate content 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 (HPR) catalyzes the reaction between the probe and hydrogen peroxide. The intensity of the color is measured at a wavelength of 540 nm. The concentration of Pyruvate in the sample is then determined by comparing the O.D. of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store Standard and 10X Blocking Buffer at ≤ -20°C.Store other component at 2-8°C at all times. Use the kit before expiration date.

Component	Quantity	Storage information
Standard (8.0 mM)	50 µL	4°C
Assay Buffer	25 ml	4°C
Probe	50 µL	-20°C
HRP-Streptavidin Solution	10 µL	-20°C
Flavin Adenine Dinucleotide (FAD)	50 µL	-20°C
Thiamine Pyrophosphate (TPP)	50 µL	-20°C
Pyruvate Oxidase	300 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 540 nm
- Flat bottomed 96-well black microplate
- 10 kDa molecular weight centrifuge spin filter
- Pipettes and pipette tips

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection.
- Store Standard and 10X Blocking Buffer at ≤ -20°C.Store other component at 2-8°C at all times. Use the kit before expiration date and avoid freeze / thaws.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to 4°C / room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C. Collect the supernatants and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Cell Lysates: Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at -80°C. Perform dilutions in PBS.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Aliquot the supernatant for storage at-80°C. Perform dilutions in PBS.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer with deionized water to yield 1X Assay Buffer. Store at 4°C.
- **Reaction Mix:** Dilute the Probe at 1:100, HRP at 1:500, Pyruvate Oxidase at 1:16.7, FAD at 1:100, and TPP at 1:100 in 1X Assay Buffer. For 20 assays, mix 10 µL Probe, 2 µL HRP, 60 µL of Pyruvate Oxidase, 10 µL of FAD, and 10 µL of TPP to 908 µL 1X Assay Buffer for total volume of 1 mL. Store the Reaction Mix at 4°C for 1 day.
- **Negative Control Reaction Mix:** Dilute the Probe at 1:100, HRP at 1:500, FAD at 1:100, and TPP at 1:100 in 1X Assay Buffer (without Pyruvate Oxidase). For 20 assays, mix 10 µL Probe, 2 µL HRP, 10 µL FAD, and 10 µL TPP to 968 µL 1X Assay Buffer for total volume of 1 mL. Store the Reaction Mix at 4°C for 1 day.
- **Standards**: Add 5 µl of 8.0 mM stock standard into 495 µl 1X PBS to generate a standard with 80 µM of Pyruvate. Dilute the standards with PBS serves as zero standard (blank standard, 0 µM). The example of the standards dilution table is as below:

Standard	Pyruvate (µM)	Volume of PBS (µL)	Volume of Pyruvate (µL)
S1	80	495	5 (8.0 mM stock)
S2	40	250	250(S1)
S3	20	250	250(S2)
S4	10	250	250(S3)
S5	5	250	250(S4)
S6	2.5	250	250(S5)
S7	1.25	250	250(S6)
S8	0	250	0

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

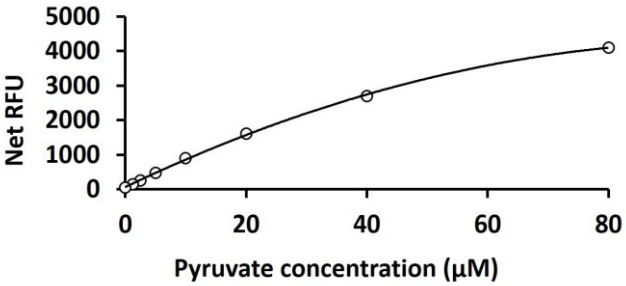
1. Add **50 µl** of **standard** and **sample** to each wells.
2. Add **50 µL** of **Reaction Mix** to **standard** and **half of sample** wells.
3. Add **50 µL** of **Negative Control Reaction Mix** to **other half of sample** wells.
4. Mix well and Incubate for 30 min at 37°C.

   Note: This assay is continuous (not terminated), therefore may be measured at multiple time points to follow the reaction kinetics.
5. Read O.D. with a microplate reader at 540 nm (500-570) nm immediately.

EXAMPLE OF TYPICAL STANDARD CURVE

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.

*Net RFU = (RFU+PO)- (RFU-PO)



Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of pyruvate present in the sample.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Pyruvate ranged from 1.25-80 µM. The mean MDD was 1.0 µM