Signosis, Inc.
Innovative Plate Assay Solutions

Pyruvate Assay Kit

Catalog # EA-7020 **(For Research Use Only)**

Introduction

The Pyruvate Assay Kit utilizes an enzyme catalyzed oxidation reaction to measure pyruvate levels in samples. First, pyruvate is oxidized by pyruvate oxidase to form a pyruvate byproduct and hydrogen peroxide. Then, the pyruvate level in the sample is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

Materials Required but Not Provided

- PBS
- 96-well clear microplate for absorbance reading or 96-well black microplate with clear bottom for fluorescence reading
- Microplate reader capable of measuring absorbance at 560 nm or fluorescence at 530nm/590nm

Materials Provided

- 10mM FAD (-80ºC)
- Probe Reagent (-20ºC)
- HRP Reagent (4ºC)
- 20mM Pyruvate Standard (-20°C)
- POX Enzyme Stock (-80ºC)

****Spin down small tubes before starting experiment. ****

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. Wash the cells once with PBS before lysing the cells.
2. For a 96-well culture plate, add 40 µL of Lysis buffer to each well and incubate at room temperature for 10 minutes.
3. Pipette the Lysis buffer up and down to detach the cells and transfer the cell lysates into a new tube.
4. If necessary, homogenize the cell lysates with a sonicator.
5. The cell lysates may be assayed directly or stored at -80°C.

Tissue Sample Preparation

1. Weigh tissue sample and add 1 mL of Tissue Lysis buffer per 100mg of tissue.
2. Homogenize the tissue samples with a tissue grinder.
3. If necessary, further homogenize the tissue samples with a sonicator.
4. Centrifuge the sample at 10,000 RPM for 5 minutes to pellet the tissue debris.
5. Collect the supernatant and measure the protein concentration of the supernatant. The tissue sample can be assayed directly or stored at -80°C.
6. Use the Dilution buffer to dilute the tissue sample to the appropriate concentration for each assay.

Pyruvate Measurement

1. Standard curve preparation: First, take eight new tubes and label them 1-8. In tube 1, prepare a 2000 µM pyruvate standard by mixing 10 µL of the provided 20 mM pyruvate standard with 90 µL of PBS. Then, for tubes 2-8, add 50 µL of PBS to each tube. Transfer 50 µL of the 2000 µM pyruvate standard from tube 1 to the PBS in tube 2 and mix to make a 1000 µM pyruvate standard. Then, transfer 50 µL of the 1000 µM pyruvate standard from tube 2 to the PBS in tube 3 and mix to make a 500 µM pyruvate standard. Continue the serial dilutions until tube 7 is done. Leave tube 8 untouched as the PBS negative control.

Standard#	Pyruvate Concentration (µM)
1	2000
2	1000
3	500
4	250
5	125
6	62.5
7	31.25
8	0

2. Reaction mix preparation: calculate the amount of each reagent needed to make the reaction mix according to the table below.

Component	Reaction Mix (per well/sample)
10mM FAD	0.05 µL
POX Enzyme	0.05 µL
PBS	49.9 µL
Total	50 µL

3. Any unused enzyme stock can be stored at -80°C for future use.
4. Add 50 µL of reaction mix to each well of the plate.
5. Add 5 µL of sample or standard to each well with reaction mix and mix thoroughly.
6. Cover the plate and incubate at room temperature for 30 minutes.
7. Detection mix preparation: calculate the amount of each reagent needed to make the detection mix according to the table below.

Component	Detection Mix (per well/sample)
Probe Reagent	0.5 µL
HRP Reagent	1 µL
PBS	48.5 µL
Total	50 µL

8. Add 50 µL of detection mix to each reaction well in the plate. **Be sure to add the detection mix quickly, since the signal begins to develop when the reagents are added. Use a multichannel pipette if possible.**
9. Cover the plate and incubate at room temperature away from light for 10-20 minutes.
   Exposure to light will produce background signal in wells
10. For a stronger signal, the plate can be incubated for another 30-60 minutes away from light.
11. Measure the absorbance of the plate at 560 nm using a plate reader. Alternatively, measure the fluorescence of the plate in a fluorescence plate reader Ex/Em 530nm/590nm.