Pyruvate Assay Kit

Catalog No.: abx097982

Size: 100 Assays

Storage: Store all kit components at 4°C.

Application: For quantitative detection of Pyruvate concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.5 µg/ml – 50 µg/ml

Introduction: Pyruvic acid (PA) is an alpha-keto acid with a carboxylic acid and a ketone functional group. Its conjugate base, pyruvate, is an important molecule that is present at the intersection of multiple biochemical pathways. It functions as a key molecule in energy production and as an antioxidant. As a product of fermentation, pyruvic acid can be found in large quantities, especially in dark beers. The concentration of pyruvic acid in blood and urine is a useful clinical marker for the various medical conditions.

Abbexa's Pyruvate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating pyruvate concentrations. Pyruvic acid reacts with 2,4-dinitrophenylhydrazine to generate a reaction product with an absorption maximum at 520 nm. The intensity of the color is proportional to the concentration of pyruvate, which can then be calculated.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Dye Reagent: 3 ml
4. Reaction Buffer: 10 ml
5. Standard: 1 vial
6. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (520 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar
9. Water bath

Protocol

A. Sample Preparation

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

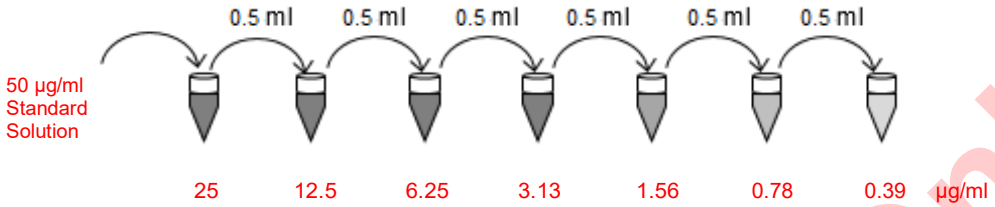
- **Serum and Plasma samples**
  To 0.1 ml of serum and plasma, add 1 ml of Assay buffer on ice. Centrifuge at 8,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 7 tubes with 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.13 µg/ml, 1.56 µg/ml, 0.78 µg/ml and 0.39 µg/ml. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 50 µg/ml Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 75 µl of sample to the sample wells.
4. Add 75 µl of prepared standards to the standard wells.
5. Add 75 µl of distilled water to the blank wells.
6. Add 25 µl of Dye Reagent to all wells.
7. Tap the plate gently to mix. Allow to stand for 2 minutes.
8. Add 100 µl of Reaction Buffer to all wells.
9. Tap the plate gently to mix. Read and record absorbance at 520 nm.

C. Calculations

Pyruvate (PA) concentration per g of sample:

$$\mathbf{Pyruvate\ (\mu g/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{50}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Pyruvate (PA) concentration per 10^4 cells or bacteria:

$$\mathbf{Pyruvate\ (\mu g/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{V_{Sample} \times N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{50}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

Pyruvate (PA) concentration per ml serum or plasma:

$$\mathbf{Pyruvate\ (\mu g/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \mathbf{50} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Standard}}$	Concentration of highest standard (50 µg/ml)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.075 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.075 ml)