Pyruvate(PA) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0473

Size:100T/96S

Components:

Extract: Liquid 100 mL ×1. Storage at 4℃.

Solution I: Liquid 5 mL×1. Storage at 4℃.

Solution II: Liquid 25 mL ×1. Storage at 4℃.

Sodium pyruvate standard solution: Liquid 1 mL ×1, 1 mg/mL, storage at 4℃.

Product Description

Pyruvate connects glucose, fatty acid and amino acid metabolism through acetyl CoA and plays an important pivotal role.

Pyruvate reacts with 2, 4-dinitrophenylhydrazine to produce pyruvate -2, 4-dinitrophenylhydrazone, which is fuchsia-red in alkaline solution.

Reagents and Equipment Required but Not Provided.

Table centrifuge, transferpettor, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, ice, mortar/homogenizer and distilled water.

Procedure

I. Extraction of Pyruvate:

1. Bacteria or cells: collect bacteria or cells into the centrifuge tube, and discard the supernatant after centrifugation. According to the bacteria or cells (10^4): the Extract volume (mL) is 500-1000:1. (It is recommended that add 1 mL of the Extract to 5 million bacteria or cells). Ultrasound breaks up bacteria or cells (power 20% or 200W, ultrasonic of 3s, interval of 10s, repeat 30 times). Stand for 30 minutes. Centrifuge at 8000 g, RT for 10 minutes. Take the supernatant for test.

2. Tissue: according to the tissue weight (g): the Extract volume (mL) is 1:5-10. (It is recommended that add 1 mL of Extract to 0.1 g tissue). Homogenate in ice bath, stand for 30 minutes, then centrifuge at room temperature, 8000 g for 10 minutes. Take the supernatant for test.

3. Serum (plasma) sample: according to the serum (plasma) volume: the Extract is 1:5-10. (It is recommended that add 1 mL of Extract into 0.1 mL of serum (plasma), then homogenate in ice bath, stand for 30 minutes. Centrifuge at 8000 g, RT for 10 minutes. Take the supernatant for test.

4. Preparation of standard: dilute standard with distilled water to 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0 μg/mL.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 520 nm and set the counter to zero with distilled water.
2. Add 75 μL standard solution or samples and 25 μL Solution I in the micro glass cuvette or 96 well flat-bottom plate, mix thoroughly. Stand for 2 minutes, then add 125 μL Solution II. Mix thoroughly. Determination of absorbance A at 520 nm.

III. Calculation of Pyruvate content:

1. Establish the standard curve according to the standard concentration and the measured value; y is the sodium pyruvate content (μg/mL), x is the absorption value.
2. Calculate by volume of serum (plasma)

Pyruvate content (μg/mL)=(y×V1)÷[(V3×V1÷(V2+V3)] =y×11

3. Calculate by protein concentration

Pyruvate content (μg/mg prot)=(y×V1)÷(V1×Cpr) =y ÷Cpr

4. Calculate by sample weight

Pyruvate content (μg/g fresh weight)=(y×V1)÷(W×V1÷V2)=y÷W

5. Calculate by bacterial or cell density

Pyruvate content (μg/10^4 cell)＝(y×V1)÷(500×V1÷V2)= y÷500

V1: Sample volume, 0.075 mL;
V2: Extract solution volume, 1 mL;
V3: Serum (plasma) volume, 0.1 mL;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
500: Total number of bacteria or cells, 5 million.

Note:

If the absorbance value exceeds the linear range, the sample size can be increased or diluted before the determination.

Recent Product Citation：

[1] Yao R, Yang Y, Lian S, et al. Effects of acute cold stress on liver O-GlcNAcylation and glycometabolism in mice[J]. International journal of molecular sciences, 2018, 19(9): 2815.

[2] Meixi Peng,Dan Yang,Yixuan Hou,et al. Intracellular citrate accumulation by oxidized ATM-mediated metabolism reprogramming via PFKP and CS enhances hypoxic breast cancer cell invasion and metastasis. Cell Death and Disease. March 2019;(IF5.959)

[3] Xiaofen Fu,Pengsong Li,Lei Zhang,et al. Understanding the stress responses of Kluyveromyces marxianus after an arrest during high-temperature ethanol fermentation based on integration of RNA-Seq and metabolite data. Applied Microbiology and Biotechnology. March 2019;103(6):2715-2729.(IF3.67)

[4] Luo M,Luo Y, Mao N,et al. Cancer-Associated Fibroblasts Accelerate Malignant Progression of Non-Small Cell Lung Cancer via Connexin 43-Formed Unidirectional Gap Junctional Intercellular Communication. Cellular Physiology and Biochemistry. November 2018

References：

[1] Venkatesh C, Ramalingam K. Lactic acid, pyruvic acid and lactate/pyruvate ratio in the Anoplocephalid tapeworm Stilesia globipunctata infecting sheep (Ovis aries)[J]. Veterinary parasitology, 2007, 144(1-2): 176-179.

Related Products：

NA0809/NA0567 Hexokinase(HK) Activity Assay Kit
NA0826/NA0584 Pyruvate Kinase(PK) Activity Assay Kit
NA0827/NA0585 Phosphofructokinase(PFK) Activity Assay Kit
NA0710/NA0469 Phosphoglycerate Kinase(PGK) Activity Assay Kit

Technical Specifications:

Detection limit: 0.1510 μg/mL
Linear range: 0.78125-50 μg/mL