PRODUCT INFORMATION & MANUAL

Pyruvate Kinase Activity Assay Kit (Colorimetric)

NBP3-24533

For research use only.
Not for diagnostic or therapeutic procedures.

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Pyruvate Kinase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24533

Method: Colorimetric method

Specification: 96T (Can detect 96 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.08 U/L

Detection range: 0.08–6.79 U/L

Average intra-assay CV (%): 5.0

Average inter-assay CV (%): 7.0

Average recovery rate (%): 103

- This kit is for research use only.
- Instructions should be followed strictly, changes of operation may result in unreliable results.
- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure pyruvate kinase (PK) activity in serum, plasma, tissue and cell samples.

▲Detection principle

Pyruvate kinase (PK), also known as phosphotransferase or phosphopyruvate kinase, is a key enzyme in the glycolysis pathway, which can catalyze the conversion of phosphoenolpyruvate to enolpyruvate and the production of ATP, so it plays an important physiological role.

In the presence of adenosine diphosphate (ADP), pyruvate kinase (PK) catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, Lactate dehydrogenase catalyzes the reaction of pyruvic acid with NADH to produce lactic acid and NAD^+. The activity of pyruvate kinase can be calculated by detection the absorbance changes at 340 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	10 mL × 2 vials	-20℃ , 12 months
Reagent 2	Substrate A	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 3	Substrate B	Powder × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Solution	1.2 mL × 2 vials	-20℃ , 12 months, shading light
	UV Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Test tube, Micropipettor, Vortex mixer, Incubator, Microplate reader (330-350 nm)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The sample for detection is better to be as fresh as possible, as the activity of PK will be reduced during long-term storage.
2. The time of A_2 can be extended if the value of serum sample is too low.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 powder with 1.2 mL double distilled water. Preserve it on ice with shading light for use and can be stored at -20℃ for 3 days with shading light.
3. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 powder with 10 mL reagent 1. The prepared solution can be stored at -20℃ for 3 days with shading light .
4. Preparation of enzyme working solution:
   Mix reagent 2 working solution and reagent 4 at a ratio of 1:1. Prepare the needed fresh solution before use and store it with shading light. The prepared solution should be used up on the same day.

▲ Sample preparation

1. Serum and plasma samples:

Detect directly (If the sample is turbid, centrifuge at 12000 g for 10 min before detection).

2. 10% tissue homogenate sample:

Weigh the tissue accurately and add normal saline (0.9% NaCl) at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4℃ , then take the supernatant for measurement. The supernatant after centrifugation must be clarified, and if there is turbidity, it must be centrifuged again. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Collect the 1×10^6 cells, add 0.2 mL normal saline (0.9% NaCl). Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.08–6.79 U/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
10% Rat kidney tissue homogenate	4-6
10% Rat lung tissue homogenate	4-6
10% Rat liver tissue homogenate	4-6
10% Rat brain tissue homogenate	4-6
10% Mouse liver tissue homogenate	4-6
10% Mouse heart tissue homogenate	4-6
Rat serum	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
Human serum	1
Dog plasma	1
HL-60 cell	1
293T cell	1

Note: The diluent is normal saline (0.9% NaCl).

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

Note:S1-S96, sample wells.

▲Detailed operation steps

(1) Sample well: Add 10 μL of sample to the wells.

(2) Add 150 μL of reagent 3 working solution and 40 μL of enzyme working solution into each well.

(3) Measure the OD value of each well at 20 s and 3 min 20 s respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\Delta A = A_1 - A_2$.

▲Summary operation table

	Sample well
Sample (μL)	10
Reagent 3 working solution (μL)	150
Enzyme working solution (μL)	40
Measure the OD value of each well at 20 s and 3 min 20 s respectively, recorded as A_1, A_2, $\Delta A = A_1 - A_2$.	

▲Calculation

1. Serum/plasma samples:

Definition: the enzyme amount of 1 mmol of NADH consumed by 1 L of liquid sample per minute at room temperature is defined as 1 unit.

$$\text{PK activity (U/L)} = \Delta A_{340} \div (6220 \times d) \times V_{total} \div V_{sample} \div T \times f \times 1000$$

2. Tissue and cell samples:

Definition: the enzyme amount of 1 mmol of NADH consumed by 1 g sample protein per minute at room temperature is defined as 1 unit.

$$\text{PK activity (U/L)}$$

$$= \Delta A_{340} \div (6220 \times d) \times V_{total} \div (V_{sample} \times C_{pr}) \div T \times f \times 1000$$

Note:

ΔA_{340}: $A_1 - A_2$.

6220: The molar extinction coefficient of NADH, L/mol•cm

d: Optical path, 0.6 cm

V_{Total}: The total volume of the reaction system, 0.2 mL.

V_{Sample}: The volume of the sample, 0.01 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

T: The time of reaction, 3 min.

f: Dilution factor of sample before test.

1000: 1 mol/L=1000 mmol/L

Appendix I Data

▲Example analysis

For rat liver tissue, take 10 μL of 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation table.

The results are as follows:

the A_1 of the sample is 1.043, after 3 minutes of reaction, the A_2 of the sample is 0.892, the concentration of protein in sample is 8.95 gprot/L, and the calculation result is:

PK activity (U/gprot)

= (1.043 – 0.892) ÷ (6220 × 0.6) × 0.2 ÷ (0.01 × 8.95) ÷ 3 × 5 × 1000 = 0.15 U/gprot