

# PYRUVATE KINASE (PK)

KINETIC DETERMINATION IN SERUM AND ERYTHROCYTES  
 UV METHOD

For in vitro diagnostic use only

Kit: 20 x 3 mL

Cod. PK8830

## PRINCIPLE

PK in the sample catalyzes the reaction between ADP and PEP (phosphoenolpyruvate), forming Pyruvate. The Lactate dehydrogenase (LDH) in the system catalyzes the reaction between Pyruvate and NADH, giving Lactic ac. and NAD. The decrease of absorbance in 10 min at 340 nm, due to the oxidation of NADH is proportional at the activity of the PK in the sample.

## REAGENTS

Components of the kit:		<b>Cod. PK8830</b>
<b>*REAGENT 1</b> (liquid)		<b>1 x 60 mL</b>
Good Buffer	>20 mmol/L	
<b>*REAGENT 2</b> (Iyo)		<b>20 x 3 mL</b>
NADH	>0.1 mmol/L	
PEP	>0.1 mmol/L	
LDH	> 1000 U/L	
<b>*REAGENT 3</b> (Iyo)		<b>3 x 2 mL</b>
ADP	>0.1 mmol/L	

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

## PREPARAT. OF THE WORKING REAGENT

### \*KIT 20 x 3 ml (Cod. PK8830)

Add 3 ml of \*Reagent 1 to one vial of \*Reagent 2.  
 Mix gently until dissolution.

STABILITY: the reconstituted \*Reagent 2 at 2-8°C in a dark place, is stable up to 3 days, if **not contaminated during handling**.

Add 2 ml of DISTILLED WATER to one vial of \*Reagent 3.  
 Mix gently until dissolution.

STABILITY: the reconstituted \*Reagent 3 at 2-8°C in a dark place, is stable up to 2 weeks, if **not contaminated during handling**.

Close immediately after handling.

The Reagents have to be used properly, to avoid contamination. Incompetent handling will keep us harmless from any responsibility.

## SAMPLE COLLECTION, STORAGE and

### PRE-TREATMENT

- No haemolyzed fresh serum, without other treatments.  
 To test immediately.

**A 50% of activity may be lost after 24 h at 2-8°C.**

- Plasma: we have no data about their use.
- Whole blood collected with EDTA, heparin or ACD (Acid-Citrate-Dextrose) may be used to stabilize and store Pyruvate Kinase for about 15 days at 2-8°C, before to prepare the haemolyzate to test. (Beutler, E. in REFERENCES)
- Erythrocytes 1<sup>st</sup> step: you must determine the number of erythrocytes in the normal way, reporting the result  
 $En = n \times 10^9$  erythrocytes/ml blood for each sample.

- Erythrocytes 2<sup>nd</sup> step: prepare the haemolyzate.  
 Wash 0,2 ml of whole blood with 2 ml of saline.  
 Centrifuge 3000 rpm x 10 min. Eliminate the supernatant and resuspend with 2 ml of saline. Repeat 3x.  
 After the elimination of the last supernatant, the washed and precipitated by centrifugation Erythrocytes must be suspended with 2,0 ml of DISTILLED WATER.  
 Leave to wait 15 min x 2-8°C.  
 Then centrifuge 3000 rpm x 10 min.

The supernatant is the HAEMOLYZATE. To test immediately.  
 A 50% of activity may be lost after 24 h at 2-8°C.

## PROCEDURE

- Wavelength: 340 nm
- Pathlength: 1 cm
- Reading: against Reagent Blank
- Temperature: 25-37°C
- Method: kinetic
- Reaction: 5 + (10+10) minutes up to 90 U/L at 37°C up to 52 U/L at 25°C
- Linearity (on serum): 1/5/0,2 (serum) 1/29/1 (erythrocytes)
- Sample/Reagent:

Let reagent reaches the working temperature before using.

Pipette into a test tube or cuvette labelled:

S: Sample (S=serum, E=erythrocytes); BL (Blank):

	BL	S	E
Working Reagent (*R1+*R2)	2500 µl	2500 µl	2500 µl
Serum Sample	----	500 µl	----
DISTILLED WATER	500 µl	----	400 µl
<b>Erythrocytes Sample</b>	----	----	<b>100 µl</b>

Mix kindly and put in stand-by at the right temperature.  
 Wait exactly 5 min.; make the FIRST reading (E1 and Ebl1).  
 Put again in stand-by at the right temperature.  
 Wait exactly 10 min.; make the SECOND reading (E2 and Ebl2).  
 Add:

Starter (*Reagent 3)	100 µl	100 µl	100 µl

Mix kindly and make the THIRD reading (E3 and Ebl3).  
 Put in stand-by at the right temperature.  
 Wait again 10 min.; make the FOURTH reading (E4 and Ebl4).  
 Determine the diff. of absorbance for 10 minutes due to PK activity:

$$\text{SERUM } \Delta A(S) (10 \text{ min.}) = (E3 - E4) - (E1 - E2)$$

$$\text{ERYTHROCYTES } \Delta A(E) (10 \text{ min.}) = (E3 - E4) - (E1 - E2)$$

$$\text{BLANK } \Delta Abl (10 \text{ min.}) = (Ebl3 - Ebl4) - (Ebl1 - Ebl2)$$

Subtract the Blank ( $\Delta Abl$ ) from the samples:

$$\text{SERUM } \Delta A (10 \text{ min.}) = \Delta A(S) (10 \text{ min.}) - \Delta Abl (10 \text{ min.})$$

$$\text{ERYTHROCYTES } \Delta A (10 \text{ min.}) = \Delta A(E) (10 \text{ min.}) - \Delta Abl (10 \text{ min.})$$

## CALCULATION

Insert the value found in the following formula:

### SERUM

$$PK (U/L \text{ at the choiced temp.}) = (\text{Serum } \Delta A (10 \text{ min.})) \times 98,4$$

### ERYTHROCYTES

$$PK (mU/10^9 \text{ erythrocytes/ mL at the choiced temp.}) =$$

$$= U/10^9 \text{ erythrocytes/ L at the choiced temp.}) =$$

$$= (\text{Erythrocytes } \Delta A (10 \text{ min.})) \times 492,1 \times 10 (\text{dil. Factor}) \times 1/n$$

$$= \Delta A (10 \text{ min.}) \times 492,1 \times 10 (\text{dil. Factor}) \times 1/n$$

where n = erythrocytes number mentioned in 10<sup>9</sup>.

For instance:

$$\text{If we have an Erythrocyte sample } 5,3 \times 10^9 \text{ erythr./ mL with}$$

$$\Delta A (E) (10 \text{ min.}) = (E3 - E4) - (E1 - E2) = (0,350) - (0,018) = 0,332$$

$$\Delta Abl (10 \text{ min.}) = (Ebl3 - Ebl4) - (Ebl1 - Ebl2) = (0,018) - (0,006) = 0,012$$

$$\Delta A (10 \text{ min.}) = \Delta A(E) (10 \text{ min.}) - \Delta Abl (10 \text{ min.}) = 0,332 - 0,012 = 0,320$$

$$\text{so PK (mU/10}^9 \text{ erythrocytes/mL)} = 0,320 \times 492,1 \times 1/5,3 = 1575/5,3$$

$$= 297 \text{ mU/10}^9 \text{ erythrocytes/mL.}$$

But the PK in erythrocytes may be also expressed as U/g Hb.

At first must be determined the concentration of Hemoglobin in g/dL. After that use this formula

$$\text{PK (U/g Hemoglobin)} = \Delta A (10 \text{ min.}) \times 492,1 \times 10(\text{dil. Factor}) \times 1/\text{Hb(g/dL)}$$

Where:  
Hb (g/dL) = Hemoglobin conc. determined for each sample

For instance:

If we have an Erythrocyte sample at an hemoglobin concentration of 14,2 g/dL, with

$$\Delta A (E) (10 \text{ min.}) = (E3 - E4) - (E1 - E2) = (0,350) - (0,018) = 0,332$$

$$\Delta \text{Abl} (10 \text{ min.}) = (\text{Ebl3} - \text{Ebl4}) - (\text{Ebl1} - \text{Ebl2}) = (0,018) - (0,006) = 0,012$$

$$\Delta A (10 \text{ min.}) = \Delta A(E) (10 \text{ min.}) - \Delta \text{Abl} (10 \text{ min.}) = 0,332 - 0,012 = 0,320$$

$$\text{so PK (U/g Hemoglobin)} = 0,320 \times 4921 \times 1/14,2 = 1575/14,2$$

= **110,9 U/g Hemoglobin.**

## REFERENCE VALUES

### Serum

	37°C	25°C
U/L	0 - 45	0 - 26

### Erythrocytes

	37°C	25°C
mU/ 10 <sup>9</sup> erythrocytes/ml	111 - 406	60 - 220
U/g Hemoglobin	41 - 152	22 - 82

It is suitable that every laboratory determine its reference values.

## PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

**Linearity:** SERUM till 90 U/L (37°C)  
ERYTHROCYTES till 900 mU/ 10<sup>9</sup> erythrocytes/ml

**Sensitivity:** The minimum detectable is

SERUM till 2 U/L (37°C)  
ERYTHROCYTES till 20 mU/ 10<sup>9</sup> erythrocytes/ml

### Within-run Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	20,1 ± 1,11	2,8
Serum 2	54,8 ± 2,72	2,5

### Run-to-run (Day-to-day) Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	20,1 ± 1,74	4,3
Serum 1	54,9 ± 3,11	2,8

**Interferences:** See References point 3.

**Correlation:** A group of 20 sample was assayed by this procedure and using a similar Reagent. Comparison of the data gave following results:

Linear regression equation  $y = 0,9803x + 1$   
Correlation coefficient  $r = 0,9964$   $n=20$

## NOTE

1. A proportional variation of reaction volumes do not modify the result.
2. We suggest do not mix Reagents from different Production lots.
3. Dilute 1:10 with Saline the samples with activity higher than 90 U/L serum) at 37°C; repeat the tests and multiply the results x10.
4. PAY ATTENTION!

Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.

5. Very deep attention must be given to interfering substances: a few drugs and other substances are able to influence levels of PK (see References 2).

7. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.

8. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

## REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975)
3. Beisenherz G. et al., Z. Naturforsch. 8b, 555 (1953)
4. Beutler E., Red Cell Metabolism, Grune & Stratton, New York (1971).

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