



PYRUVATE (PYRUVIC ACID)

UV DETERMINATION IN WHOLE BLOOD
For in vitro diagnostic use only

Kit: 10 x 10 mL

Cod. PY8825

SUMMARY

Pyruvate levels are very important, because give an information about circulatory disease. The increase of pyruvate levels are mentioned in several disease as: diabetes mellitus, liver disease, neoplastic disorder, muscular dystrophy and congestive heart failure.

PRINCIPLE

The Lactate Dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate with simultaneous oxidation of NADH to NAD. The decrease of absorbance of NADH, is directly proportional to the pyruvate concentration in the sample.

REAGENTS

Components of the kit:	Cod. PY8825
*REAGENT 1 (liquid)	3 x 70 mL
*REAGENT 2 (Iyo)	10 x 10 mL
*REAGENT 3 (suspension)	1 x 5 mL
*REAGENT 4 Standard (liquid)	1 x 10 mL

Pyruvate	(4,0 mg/dL = 40 mg/L = 0,45 mmol/L)
Good Buffer	>400 mmol/L
LDH	>250 KU/L
NADH	>0,1 mmol/L

Activators, Stabilizers

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

AUXILIARY REAGENTS (Not supplied with this kit)

To assure proper test performance, we suggest following kits:
- SUBSTRATE Elevated Control (Iyo) 6 x 2 mL Cod. SCE3005
- SUBSTRATE Low Control (Iyo) 6 x 2 mL Cod. SCL3006
 that contain a few Substrates at two different levels of concentration (see the inserts). The controls cod.SCE3005 and SCL3006 must be used as the samples, that means they will be deproteinized.

SAMPLE

• Whole blood, deproteinized for pyruvate is recommended.

Sample Deproteinization

- Prepare protein-free supernatants as follows:
- draw BLOOD following standardized procedure: it is suggested that patient be in a fasting and resting state.
 - into a centrifuge tube put 4,0 mL of cold 8% (w/v) perchloric acid (PCA) (prepared by diluting 7 mL of 70% (w/w) perchloric acid to 100 mL with distilled water).
 - quick pipet **2,0 mL** BLOOD into that tube and vortex mix for 30 sec. Keep cold the blood-precipitate mixture again for 5 min., to assure complete protein precipitation.
 - centrifuge for about 10 min. x 1500 g.
 - the clear PCA supernatant is ready for use.

NOTE: - a second centrifugation of the supernatant may be necessary to obtain a clear protein-free solution.
 - all the operation must require a short time; pyruvate in PCA is stable for a few weeks refrigerated.

PREPARAT. OF THE WORKING REAGENT (*R1+*R2)

***KIT 10 x 10 ml (Cod. PY8825)**

Add 5 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution.

STABILITY: 7 days at 2-8°C.

Mix kindly and let the working reagent reaches the working temperature before use. Close immediately after handling.

PREPARATION *REAGENT 3

***Reagent 3 REAGENTS READY TO USE.**

Let the reagents reach the working temperature before use.

Mix kindly before use. Close immediately after handling.

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

PROCEDURE FOR WHOLE BLOOD (deproteinized) (procedure for CALIBRATION is different and follows)

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against Water
- Temperature: 37°C
- Method: end-point
- Reaction: 5+5 minutes
- Linearity: up to 3 mg/dL (0,340 mmol/L)
- Sample/Reagents: 4/1/1/0,1

Let reagent reaches the working temperature before using.

Pipette in a test tube or cuvet so labelled : S: Sample:

	S
PCA supernatant fluid (from sample deproteinization)	2000 µl
*Reagent 1	500 µl

Mix kindly but surely: the mixture must be at the right pH **before** adding WORKING REAGENT. Incubate for 5 min. x 37°C and add:

Working Reagent	500 µl
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Read for the first time the absorbance of sample (As1) against Water (INITIAL A). Immediately after this one, add:

*Reagent 3	50 µl
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Mix kindly. Exactly 5 min. after the first reading, make the second reading of sample (As2) (FINAL A).

Procedure for PYRUVATE CALIBRATION

Preparation of the CALIBRATION REAGENT (*R1+*R2)

***KIT 10 x 10 ml (Cod. PY8825)**

Add 10 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution. STABILITY: 7 days at 2-8°C.

Mix kindly and let the working reagent reaches the working temperature before use. Close immediately after handling.

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

- Wavelength: 340 nm (334-365 nm)
- Pathlength: 1 cm
- Reading: against Water
- Temperature: 37°C
- Method: end-point
- Reaction: 5+5 minutes
- Linearity: up to 3 mg/dL (0,340 mmol/L)

Let reagent reaches the working temperature before using.

Pipette in 5 test tubes or cuvetts so labelled :

Cuvet N°	Calibrat. Reagent	Water	*Reagent 4	BLOOD PYRUVATE	
				(mg/dL)	(mmol/L)
1	1000 µl	1900 µl	100 µl	0,600	0,068
2	1000 µl	1800 µl	200 µl	1,200	0,136
3	1000 µl	1700 µl	300 µl	1,800	0,204
4	1000 µl	1600 µl	400 µl	2,400	0,272
5	1000 µl	1500 µl	500 µl	3,000	0,340

Mix kindly and incubate for about 5 min. at 37°C.

Read for the first time the absorbance of sample (As1) against Water (INITIAL A). Immediately after this one, **add to the 5 cuvetts:**

*Reagent 3	50 µl
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Mix kindly. Exactly 5 min. after the first reading, make the second reading of sample (As2) (FINAL A).

CALCULATION

Plot each value found on the Calibration Curve. The Calibration Curve has to be always repeated for each new lot of Reagent.;

or

$$(As1 - As2) \times 6,29 = \text{mg/dL of Pyruvic Acid}$$

$$(As1 - As2) \times 0,714 = \text{mmol/L of Pyruvic Acid}$$

$$\text{Pyruvic Acid in mmol/L} = 0.114 \times \text{mg/dL of Pyruvic Acid}$$

REFERENCE VALUES

Pyruvic Acid 0,3 - 0,7 mg/dL (0,030 – 0,080 mmol/L)
(in fasting Venous Blood)

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: The Pyruvate Reagent is linear up to 3 mg/dL (0,34 mmol/L).
For concentrations ≥ 3 mg/dL, dilute the sample 1:2 with saline sol., repeat the determ. and multiply the result $\times 2$.

Sensitivity: The minimum detectable is 0,1 mg/dL.

Within-run Precision:

	Mean (mg/dL) $\pm 2s$	CV %
Sample 1	0,4 \pm 0,05	4,7
Sample 2	2,6 \pm 2,3	1,7

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

	Mean (mg/dL) $\pm 2s$	CV %
Sample 1	0,4 \pm 0,06	4,9
Sample 2	2,5 \pm 2,5	1,9

Interferences: See References point 2.

Correlation: A group of 20 samples from 0,3 to 2,8 mg/dL was assayed by this procedure and using a similar commercially available Pyruvate Reagent. Comparison of the data gave following results:

Linear regression equation $y = 1,0098x - 0,12$
Correlation coefficient $r = 0,9877$

NOTE

1. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Pyruvate (see References 2.).
2. A proportional variation of reaction volumes do not modify the result.
3. For concentrations of Pyruvate ≥ 3 mg/dL (0,340 mmol/L), dilute the sample 1:2 (1+1) with saline sol., repeat the determination and multiply the result $\times 2$.
4. We suggest not to mix Reagents from different Production lots.
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. 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)

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