Pyruvate in whole blood

0142 KIT E PYR

Instruction manual for spectrophotometric assay
for in vitro diagnostic use

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Document version: 1.3
Replaces: 1.2
Date of release: 12-11-2020

CE 98/79/EC - IVD Medical Devices

Contents

1. Introduction

1.1 Intended Use

This spectrophotometric kit is intended for the determination of Pyruvate in whole blood.

The components in this kit must be used as stated in the user manual.

1.2 Intended User

This kit is designed for (healthcare) laboratory professional use. Diagnotix recommends that users adhere to ISO 15189 Medical Laboratories.

1.3 Notice Regarding Serious Incidents

Following (EU) 2017/746 Annex I, Chapter III, 20.4.1 af), any serious incident that has occurred in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

1.4 IVD symbols

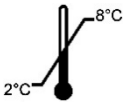
Order Number

Lot Number

For in vitro diagnostic use

See instructions for use

Manufacturer



Temperature limits

Contains sufficient for < n > tests

Expiry date

1.2 Clinical background

In aerobic metabolism the cell absorbs organic molecules such as amino acids, lipids and glucose, and breaks them down to obtain ATP. This happens to approximately 40 % of the energy released through catabolism, the rest is turned into heat. The generated ATP provides energy for synthesis (anabolism), movement, contraction, active transport and mitosis.

When there is insufficient oxygen available, lactic acidosis may develop. This can occur after strenuous exercise or prolonged tissue hypoxia as active (muscle) cells rely on anaerobic respiration producing lactate from the glucose metabolism. Tissue hypoxia is seen in severe anaemia, shock, cardiac arrest, and pulmonary insufficiency. The aerobic oxidation of pyruvic acid in the Krebs cycle is blocked, resulting in the formation of lactate. When the source of the lactate cannot be restricted tachypnoea, weakness, fatigue, stupor, and finally coma will occur. Lactic acidosis can be caused by drugs and alcohol, acquired and hereditary defects in gluconeogenesis, uraemia, liver failure, tumours, seizures, anaesthesia, and abnormal intestinal bacteria producing D-lactate.

The measurement of lactate is important in the differential diagnosis of a metabolic acidosis.

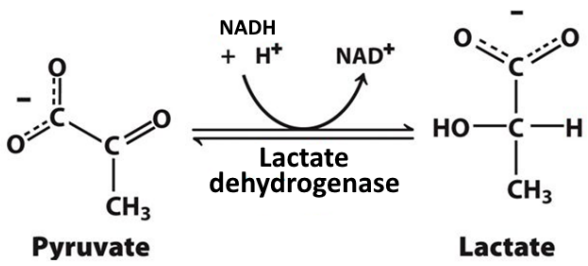
For the first screening in case of suspected mitochondrial energy metabolism disorder with persistent lactic acidosis, in addition to the analysis of lactate, the determinations of pyruvate, beta hydroxy butyric acid and acetoacetate and the lactate/pyruvate and beta hydroxybutyric acid/acetoacetate ratio are important.

Notes:

- When blood is drawn the patient should be at rest. Immediately after the blood is drawn it needs to be deproteinized with Perchloric acid (0178 PCA).
- For the correct interpretation of tissue hypoxia, liver failure and sepsis/meningitis should be excluded.

1.3 Description of the analytical procedure

In the presence of an excess of NADH, pyruvate will be converted into lactate catalysed by the enzyme lactate dehydrogenase. This yields the following reaction[1]:



In the presence of the excess of NADH essentially all pyruvate is converted into lactate. This means that an equal amount of NADH will be converted into NAD^+. The extinction measured from NAD^+ at 340 nm is much lower than the extinction from NADH at this wavelength. The concentration of pyruvate in a blood sample can therefore be calculated from the reduction of the extinction measured with this method.

[1] W.H. Freeman and Company (2012)

2. Components of the Pyruvate Kit

2.1 Ordering information

0142 KIT E PYR - Complete Kit for pyruvate in whole blood

Contents (for 150 assays):

β-HBA, Lactate & Pyruvate Calibrator Set (Calibrator 1 – 3)	0131 CAL E HLP	3 x 4 x 1 ml
Pyruvate Buffer	0145 E PYR	1 x 40 ml
Pyruvate Substrate	0146 E PYR	5 x 7 ml
Pyruvate Enzyme	0147 E PYR	1 x 5 ml

Separately available components:

β-HBA, Lactate & Pyruvate Calibrator Set (Calibrator 1 – 3)	0131 CAL E HLP	3 x 4 x 1 ml
Pyruvate Buffer	0145 E PYR	1 x 40 ml
Pyruvate Substrate	0146 E PYR	5 x 7 ml
Pyruvate Enzyme	0147 E PYR	1 x 5 ml
Perchloric acid 15% (v/v)	0178 PCA	1 x 80 ml

β-HBA, Lactate & Pyruvate Control I	0139 E HLP	10 x 1 ml
β-HBA, Lactate & Pyruvate Control II	0140 E HLP	10 x 1 ml
β-HBA, Lactate & Pyruvate Control III	0141 E HLP	10 x 1 ml
β-HBA, Lactate & Pyruvate Control Set	0132 CON E HLP	3 x 3 x 1 ml

2.2 Safety information

Several components are chemical preparations and may contain hazardous substances. For safety information, please consult the Material Safety Data Sheet (MSDS) of each component.

The raw biological material is tested for contaminants. However, because no test method can offer complete assurance that products derived from biological sources will not transmit infectious agents, it is recommended that this product be handled with the same precautions as patient samples.

2.3 Storage conditions and lifetime of kit components

Please unpack the kit components from the transport packaging *immediately upon receipt* and follow the instructions for storage conditions indicated on the product labels.

2.3.1 Calibrators and controls

0131 CAL E HLP | β-HBA, Lactate & Pyruvate Calibrator Set
0132 CON E HLP | β-HBA, Lactate & Pyruvate Control Set
0139 E HLP | Pyruvate Control I
0140 E HLP | Pyruvate Control II
0141 E HLP | Pyruvate Control III

2.3.1.1 Handling

Reconstitute and deproteinize the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute β-HBA, Lactate & Pyruvate Calibrator set and Controls distilled or deionised water using a volumetric pipette.
3. Replace the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Carefully remove the plug avoiding any loss of contents.
7. Add exactly 500 µl Perchloric acid, 15% (0178 PCA) to each vial using a volumetric pipette.
8. Replace the plug and shake vigorously.
9. Transfer the contents to marked centrifuge tubes or cups.
10. Centrifuge (5 min, 10000 x g or more).
11. Use the preparations as a patient sample.

2.3.1.2 Stability and storage

The stability of the calibrators and controls are:

Before reconstitution:	2 - 8 °C	Until expiry date printed on the product label
After reconstitution:	2 - 8 °C	48 hours
After deproteinization:	2 - 8 °C	48 hours
	- 20 °C	3 months

The declared stated stabilities are only valid in case of no bacterial contamination.

2.3.2 Buffer

0145 E PYR | Pyruvate Buffer

2.3.2.1 Handling

The Reagent is liquid and ready for use.

2.3.2.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 5 weeks if closed and stored at 2 - 8 °C

The declared stated stabilities are only valid in case of no bacterial contamination

2.3.3 Substrate

0146 E PYR | Pyruvate Substrate

2.3.3.1 Handling

Reconstitute the substrate as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Pyruvate Substrate with exactly 7 ml **Pyruvate Buffer (0145 E PYR)** using a volumetric pipette.
4. Replace the plug and let stand during 5 minutes.
5. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have been dissolved, do not shake. Avoid foaming.
8. The substrate reagent is now ready to use

2.3.3.2 Stability and storage

The stability of the Pyruvate Substrate is:

Before reconstitution:	2 - 8 °C	Until expiry date printed on the product label
After reconstitution:	2 - 8 °C	24 hours

The declared stated stabilities are only valid in case of no bacterial contamination

2.3.4 Enzyme

0147 E PYR | Pyruvate Enzyme

2.3.4.1 Handling

The Reagent is liquid and ready for use.

2.3.4.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 5 weeks if closed and stored at 2 - 8 °C

The declared stated stabilities are only valid in case of no bacterial contamination

2.3.5 Perchloric acid

0178 PCA | Perchloric acid 15% (v/v)

2.3.5.1 Handling

The Reagent is liquid and ready for use.

2.3.5.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 5 weeks if closed and stored at 2 - 8 °C

3. Required instruments

Using this test kit requires a (automated) spectrophotometer

3.1 Parameters and Conditions

3.1.1 Reagent

Substrate volume: 190 µl
Enzyme volume: 20 µl

For the preparation of the substrate see paragraph 2.3.3.1

3.1.2 Sample

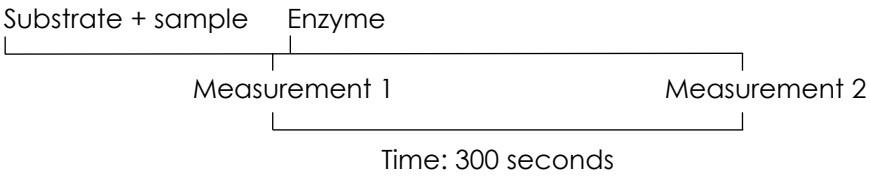
Sample volume: 30 µl

For the preparation of the blood sample see paragraph 4.2.2
For the preparation of the calibrators or controls see paragraph 2.3.1.1

3.1.3 Spectrophotometer conditions

Measurement type: Two point end point
Wavelength: 340 nm
Temperature: 37 °C
Units: mmol/l
Reagent blank: yes
Sample blank: no
Calibration points: 3
Reaction direction: decreasing
Calculation: linear regression
On board stability substrate: 1 day (if temperature is below 10 °C)
On board stability enzyme: 1 week (if temperature is below 10 °C)

Measurement:



For manual measurements, increase the reagent and sample volumes by a factor of 5.

4. Sample

4.1 Sample material

Use whole blood (EDTA-tubes)
Samples need to be deproteinized as soon as possible.

Stability after deproteinization: 1 month (- 20°C)

4.2 Sample preparation

4.2.1 Reconstitution of the lyophilised Calibrators / Controls.

See 2.3.1.1 and the product data sheets.

4.2.2 Sample preparation (whole blood, calibrator or control)

1. Prepare a centrifuge tube with 500 µl of Perchloric acid 15%.
2. Add 1 ml of blood, preferably while mixing on a vortex mixer, directly after sample collection.
3. Shake vigorously to make sure the sample is completely deproteinized.
4. Place the tube in the fridge (2 – 8 °C) for 15 minutes.
5. Centrifuge (5 min, 10000 x g or more).
6. Repeat if the supernatant is not clear.
7. The supernatant is ready to be measured.

5. Test data (Validation report)

5.1 Linearity

	mmol/l
Pyruvate	0.50 mmol/l

5.2 Limit of quantification

	mmol/l
Pyruvate	0.005

5.3 Repeatability

Item	**Measured value (mmol/l)**	**Standard Deviation (mmol/l)**	**CV (%)**	**N**
Control Level I	0.04	0.0005	1.30	20
Control Level III	0.34	0.0011	0.33	20
Patient material	0.06	0.0007	1.16	20

5.4 Reference Ranges

	mmol/l
Pyruvate	0.03 – 0.09
Lactate : Pyruvate ratio	<15

The indicated reference ranges are taken from scientific literature[2]. It is recommended that each laboratory establishes its own reference ranges.

6. References

1. W.H. Freeman and Company (2012), Biochemistry, Seventh Edition, 468
2. Dutch Association of Clinical Chemists: https://www.nvkc.nl/algemeen-overzicht-referentiewaarden

[2] https://www.nvkc.nl/algemeen-overzicht-referentiewaarden