# **ENZYMATIC POTASSIUM**

Instructions for use

Ref.: 152

Intended use . System for quantitative determination of potassium ion in serum samples by kinetic enzymatic reaction.

Professional use.

[For in vitro diagnostic use].

**Test principle**. Potassium is determined via enzymatic reaction, in which phosphoenolpyruvate is converted to pyruvate by the action of a potassium-dependent pyruvate kinase.

The pyruvate produced is converted to lactate in presence of NADH (nicotinamide dinucleotide), in a reaction which is catalyzed by lactate dehydrogenase. The oxidation of NADH to NAD and subsequent decrease in optic density at 380 nm is proportional to the amount of potassium in sample.

$$\begin{array}{c} \text{Phosphoenolpyruvate} + \text{ADP} & \xrightarrow{\text{K}^+} & \text{Pyruvate} + \text{ATP} \\ \hline \text{Pyruvate} + \text{NADH} & \xrightarrow{\text{Lactate}} & \text{Lactate} + \text{NAD} \\ \hline \end{array}$$

Summary . The Enzymatic Potassium Labtest method was developed using the specificity of the potassium-depended pyruvate kinase, being a handful alternative to flame photometry and lon-Selective Electrode (ISE) methodologies, which require specific equipment.

The reaction components are distributed in two reagents that are ready to use, which makes the liquid presentation more stable. The great analytical specificity, which is easy to use in automated analyzers capable of measuring absorbance at 380 nm, allows the user to measure the potassium ion concentration along with other biochemical routine exams, making the analytical process practical and agile.

Methodology. Enzymatic reaction

# Reagents

# 1. RIT - Reagent 1 - Ready to use - Store at 2 - 8 °C.

Lactate dehydrogenase <50 KU/L; Phosphoenolpyruvate <100 mmol/L; analogue NADH <10 mmol/L; ADP <100 mmol/L, and lithium azide < 0.095%.

#### 2. RIZ - Reagent 2 - Ready to use - Store at 2 - 8 °C.

Pyruvate kinase < 50 KU/L, and lithium azide < 0.095%.

# 3. CALII - Calibrator 1 - Ready to use - Store at 2 - 8 °C.

Check the calibrator concentration on the bottle label. Liquid preparation containing potassium ions in buffered solution 50 mM pH 7.4, cofactor, osmolytes and sodium azide 0,05%.

#### 4. CAL 2 - Calibrator 2 - Ready to use - Store at 2 - 8 °C.

Check the calibrator concentration on the bottle label. Liquid preparation containing potassium ions in buffered solution 50 mM pH 7.4, cofactor, osmolytes and sodium azide 0,05%.

The reagents must be kept out of their storage temperature only for the time necessary to get the volume that will be used in tests.

Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label. Microbial or chemical contamination may decrease reagent stability.

# Precautions and warnings

Ensure the calibrators are at room temperature prior to being used. Homogenize the calibrators gently before use.

Ensure the potassium determination be made after the sodium determination when both analytes are determined for the same sample.

It is recommended to insert a probe wash step in the automated analyzer protocol when potassium is being determined, so as to reduce cross contamination caused by dragging of reagents.

The presence of air bubbles in reagents, calibrators, or in any sample during the test execution is a common cause of error in analyte determination.

Do not mix reagents from different lots.

The usual security cares should be applied to the reagent handling. They must not be pipetted by mouth aspiration. Avoid ingestion and in case of contact with eyes, wash them with plenty of water and seek medical help.

The reagents and calibrators contain azide as preservative. Avoid ingestion. In case of contact with eyes, immediately flush eyes with plenty of water and get medical assistance.

Azide may react with lead and copper plumbing and yield highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.



# Materials required not provided

- 1. Analyzer capable of measuring absorbance accurately at 380 nm.
- 2. Qualitrol H Labtest control series.
- 3. Water bath kept constantly at 37 °C.
- **4.** Pipets to measure samples and reagents.
- 5. Timer.

# Sample

Use only serum. Do not use hemolysed samples. The analyte is stable for up to 5 days at  $2-8\,^{\circ}\text{C}$  and up to  $12\,\text{months}$  at  $-20\,^{\circ}\text{C}$ , when stored in a tube proper for sample freezing.

Ensure all samples are thawed and properly homogenized prior to being used. Do not use samples with signs of contamination or samples which underwent repeated freeze and thaw cycles.

A Standard Operating Procedure (SOP) must be created to establish adequate procedures for sample collection, preparation, and storage. The errors due to bad sampling can be more damaging than the ones which may occur during the analytical procedure.

Since no known test method can offer complete assurance that human blood samples will not transmit infectious diseases, all blood samples should be considered potentially infectious and handled accordingly.

Disposal of all biological waste material should be in accordance with local guidelines.

## Interference

Sodium ions up to 150 mmol/L, conjugated bilirubin up to 20 mg/dL, unconjugated bilirubin up to 10 mg/dL, ascorbic acid up to 10 mmol/L and triglycerides up to 1000 mg/dL do not interfere significantly in the reaction.

## **Procedure**

## Manual and Semi-Automated Systems

1. Identify 3 tubes with the following denominations: "Calibrator 1", "Calibrator 2" and "Test". Pipet, as set forth in the table below:

	Calibrator 1	Calibrator 2	Test
Reagent 1	0,600 mL	0,600 mL	0,600 mL
Sample	0,015 mL	0,015 mL	0,015 mL

- **2.** Homogenize and incubate in a water bath at 37  $^{\circ}\text{C} \pm 0.2 \,^{\circ}\text{C}$  for 5 minutes.
- 3. Adjust the zero of the photometer with distilled or deionized water and add.

Reagent 2	0,150 mL	0,150 mL	0,150 mL

**4.** Homogenize and transfer immediately to the thermostated cuvette at 37 °C  $\pm$  0.2 °C. Simultaneously trigger the timer and record the absorbance (A1) after 2 minutes.

Record absorbance (A2) after 4 minutes.

The procedure suggested above is suitable for photometer whose minimum solution volume necessary for measurement is lesser than or equal to 0.8 mL. Check whether there is the need to adjust the reaction volume to the photometer being used. Sample and reagent volumes can be changed proportionally without any performance loss, and the calculation procedure remains the same. If the volumes are reduced, pay attention to the minimum solution volume for photometric detection. Sample volumes lower than 0.01 mL are critical and should be used carefully, as they increase the measurement's imprecision.

#### Calculation

#### Manual System

Calculate the difference between absorbances 1 and 2 for each calibrator ( $\Delta$  Abs):

Calibrators		Absorbance		∆ Abs
N	Concentration (mmol/L)	Abs 1	Abs 2	Abs 1 - Abs 2
1	3.0	2.022	1.641	0.381
2	7.2	1.829	1.267	0.562

Calculate the factor according to the equation below:

[Cal. 1] = Calibrator 1 concentration

[Cal. 2] = Calibrator 2 concentration

Factor = 
$$\frac{[Cal. 2] - [Cal. 1]}{\Delta Abs Cal. 2 - \Delta Abs Cal. 1}$$

Factor = 
$$\frac{7.2 - 3.0}{0.562 - 0.381} = \frac{4.2}{0.181} = 23.2$$

Factor = 23.2

Next, calculate the intersection using the following equation:

Intersection = Factor x  $\triangle$  Abs Cal. 1 - [Cal. 1] = 23.2 x 0.381 - 3.0 = 8.83 - 3.0 = 5.83 Intersection = 5.83

To obtain the sample potassium concentration, calculate the absorbance delta of the sample and use it on the following equation:

Sample Concentration (mmol/L) = Sample  $\Delta$  Abs x factor - intersection

#### Example

Sample	Absorl	Absorbances ∆ Abs	
	Abs 1	Abs 2	Abs 1 - Abs 2
1	2.036	1.621	0.415

Sample Concentration (mmol/L) = Sample  $\Delta$  Abs x factor -intersection

Sample Concentration (mmol/L) =  $0.415 \times 23.2 - 5.83$ Sample Concentration (mmol/L) = 3.80



#### Calibration

Ensure the calibrators are at room temperature prior to being used. Homogenize the calibrators gently before use.

#### Manual and semi-automated systems

2-Point Calibration

Points 1 and 2: Calibrators 1 and 2

#### **Automated systems**

3-Point Calibration
Point 0: deionized water

Points 1 and 2: Calibrators 1 and 2

# **Calibration frequency**

when the internal quality control indicates so;

when using a new reagent lot;

when using new bottle of reagent from the same lot if a new calibration has been performed for the prior reagent bottle.

# Parameters for automated analyzers

Parameter	Application	
Reaction Type	Kinetic	
Reaction Direction	Decreasing	
Primary Wavelength	380 nm	
Secondary Wavelength	700 nm	
Temperature	37 °C	
	3 Points	
Calibration	Point 0: Deionized water	
Odiibi ation	Point 1: Calibrator 1	
	Point 2: Calibrator 2	
Calibration Model	Linear	
Sample Volume*	5 μL	
R1 Volume*	200 μL	
Incubation	300 seconds at 37 °C for R1 + sample	
R2 Volume	50 μL	
Reading 1 (Abs 1)	After 120 seconds of incubation	
Ticading 1 (Abs 1)	of R1 + sample + R2 at 37 °C	
Reading 2 (Abs 2)	After 240 seconds of incubation	
ricading 2 (ADS 2)	of R1 + sample + R2 at 37 °C	

<sup>\*</sup>The sample and reagent volumes can be modified proportionally without any loss in test performance, and the calculation procedure remains the same. In case of volume reduction it is crucial to observe the minimal necessary volume for photometric reading.

**Operating interval**. The measurement operating interval is 2.0 to 8.0 mmol/L.

Internal quality control. The laboratory must keep an internal quality control program with well-defined regulations, objectives, procedures, criteria of quality specifications and tolerance limits, corrective actions and registration of activities. Control materials should be used for measurement imprecision monitoring and determination of calibration deviation.

It is recommended to use the products Qualitrol - Labtest as internal quality control.

**Expected values**. These values should be used only for orientation purposes. Each laboratory should evaluate the transferability of the expected values to its own patient population and, if necessary, estimate its own reference interval.

Serum: 3.5 - 5.1 mmol/L

**Conversion:** SI unit (mmol/L) x1 = Conventional unit (mEq/L)

# Performance characteristics3

**Recovery studies**. A sample with potassium concentration equal to 5.6 mmol/L received different analyte amounts, and the following results were obtained:

	Recovery rate				
Initial	Added	Expected	Found	necovery rate	
5.60	1.20	6.80	6.82	100.3%	
5.60	2.40	8.0	7.96	99.6%	

The proportional systematic error estimated based upon decision level equal to 5.8 mmol/L is 0.003 mmol/L. The mean systematic error (0.05%) meets the specification for Total Error of 8.4% according to Minimum Requirements of Biological Variation<sup>5</sup>.

**Method comparison**. The proposed method was compared with the reference method of similar Labtest technology, and the following results were obtained:

	Comparative Method	Labtest Method
Samples	2	3
Concentration Range (mmol/L)	2.55 - 7.17	2.55 - 7.17
Regression equation	Labtest method = 1.0077 x Comparative - 0.0664	
Correlation coefficient	0.99	9991

Using the regression equation, the systematic error (bias) was equal to -0.67% and -0.18% for samples with concentrations of 4.62 and 6.96 mmol/L, respectively.

**Imprecision**. The imprecision studies were performed using two samples with concentrations of 4.62 mmol/L and 6.96 mmol/L.

## Imprecision - Within Run

	N	Mean	SD	(%) CV
Sample 1	80	4.62	0.073	1.58
Sample 2	80	6.96	0.084	1.20

# Imprecision - Run-to-Run

•				
	N	Mean	SD	(%) CV
Sample 1	80	4.62	0.081	1.77
Sample 2	80	6.96	0.122	1.77



The total error (random error + systematic error) estimated at decision levels equal to 4.62 mmol/L and 6.96 mmol/L are equal to 3.59% and 3.10%, respectively. The results indicate that the method meets the desired specification for Total Error ( $\leq 8.4\%$ ) based on the Minimum Analytical Quality Requirement<sup>4</sup>.

**Analytical sensitivity**. Detection limit: 0.17 mmol/L. The detection limit is equal to three standard deviations obtained with 20 measurements of a sample with potassium concentration equal to 1.92 mmol/L.

**Effect of matrix dilution**. A sample with potassium concentration equal to 8.0 mmol/L was used to evaluate the system response to matrix dilution using saline solution. Using dilution factors ranging from 1.6 to 2.1, the mean recovery found was 98%. The mean systematic error (2.0%) meets the minimum specification for total systematic error ( $\leq \pm 2.8\%$ ) based on the components of biological variation

# Notes

- **1.** The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.
- 2. The water in the laboratory to prepare reagents and use in the measurements must have resistivity ≥1 megaohm.cm, or conductivity ≤1 microsiemens/cm and silicates concentration must be < 0.1 mg/L.</p>

#### References

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#### Presentation

Product	Reference	Co	ntent
	152-1/33 Labmax 240	R 1	1 X 25 mL
		R 2	1 X 8 mL
		CAL 1	1 X 3 mL
Enzymatic Potassium		CAL 2	1 X 3 mL
Liizyiiialic rolassiuiii	152-1/33 CS 400/800	R 1	1 X 25 mL
		R 2	1 X 8 mL
		CAL 1	1 X 3 mL
		CAL 2	1 X 3 mL

Applications for automatic and semi-automatic systems are available.

The number of tests for automatic systems depends on the programmed parameters.

The volume of reagent per test in automatic applications depends on the programming parameters of each equipament.

See availability of applications with Customer Service.

# **Customer information**

# [Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label provided that the procedures and storage conditions indicated on the label and in this insert have been followed correctly.

# Labtest Diagnóstica S.A.

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# Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro Symbols used with ivd devices

$\sum$	Conteúdo suficiente para $< n >$ testes Contenido suficiente para $< n >$ tests Contains sufficient for $< n >$ tests	發	<b>Risco biológico</b> Riesgo biológico Biological risk
	Data limite de utilização (aaaa-mm-dd ou mm/aaaa) Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)	CE	Marca CE Marcado CE CE Mark
CAL	Material Calibrador Material Calibrador Calibrator Material		<b>Tóxico</b> Tóxico Poison
CAL	<b>Material Calibrador</b> Material Calibrador Calibrator Material	R	Reagente Reactivo Reagent
1	Limite de temperatura (conservar a) Temperatura limite (conservar a) Temperature limitation (store at)	•••	Fabricado por Elaborado por Manufactured by
EC REP	Representante Autorizado na Comunidade Europeia Representante autorizado en la Comunidad Europea Authorized Representative in the European Community	LOT	<b>Número do lote</b> Denominación de lote Batch code
Ţį.	Consultar instruções de uso Consultar instrucciones de uso Consult instructions for use	CONTROL	Controle Control Control
REF	Número do catálogo Número de catálogo Catalog Number	CONTROL -	Controle negativo Control negativo Negative control
	Adições ou alterações significativas Cambios o suplementos significativos Significant additions or changes	CONTROL +	Controle positivo Control positivo Positive control
IVD	Produto diagnóstico in vitro Dispositivo de diagnóstico in vitro In vitro diagnostic devíce	CONTROL	Controle Control Control
LYOPH	<b>Liofilizado</b> Liofilizado Lyophilized		Corrosivo Corrosivo Corrosive
	Período após abertura Período post-abertura Period after-opening	<b>③</b>	Uso veterinário Uso veterinario Veterinary use
ĪN	Instalar até Instalar hasta Install before	<u>~</u>	Fabricado em Elaborado en Manufactured on
2	Produto de uso único Producto de un solo uso Single use product		Ref.: 280322

