



LACTATE (LACTIC ACID) REAGENT KITS
C452-0A, C454-01, V452-12

Contents	Product No.	Package
LACTATE REAGENT KIT A	C452-0A	
LACTATE REAGENT	C454-01	6 x 25 mL
LACTATE REAGENT	C454-01	1 x 25 mL
LACTATE REAGENT	V452-12	3 x 13 mL

REAGENT PREPARATION

This reagent is packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store unopened reagents at 2-8 °C.
When stored as directed, reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: +1 203-262-0330



LACTATE (LACTIC ACID) REAGENT KITS C452-0A, C454-01, V452-12 MANUAL/AUTOMATED APPLICATION

Intended Use

For **In Vitro Diagnostic** use in the quantitative determination of Lactate in plasma and spinal fluid.

Clinical Significance

Lactate as an intermediary product of carbohydrate metabolism is present in the blood entirely as lactate ion. The Lactate blood concentration is dependent on the rate of metabolism by the liver and its production by the erythrocytes and muscle cells. During exercise, Lactate may increase significantly up to ten times higher than the normal level. Pyruvate levels increase under these conditions as well and the normal ratio of Pyruvate to Lactate is approximately 10:1. Lactate in spinal fluid normally parallels blood levels. In case of biochemical alteration in the central nervous system, Lactate changes independently of blood values. Lactate levels in spinal fluid are increased in bacterial meningitis. Also, increased values are observed in trauma, seizure, brain abscess, and multiple sclerosis.

Method Principle

Lactate, by the action of Lactate Oxidase is oxidized to pyruvate and hydrogen peroxide. The hydrogen peroxide thus produced is quantitatively determined by coupling 4-aminoantipyrine with N-ethyl-N-(2-hydroxy-3-sulphopropyl)-m-toluidine (TOOS) ⁽³⁻⁴⁾, where a quinonemine dye with maximum absorption at 550 nm is produced. The following reaction scheme illustrates the reactions that occur in this method:



LACTATE REAGENT

Each liter contains:

Buffer	
4-Aminoantipyrine (4AA)	0.15 mol
TOOS	1.2 mmol
Lactate Oxidase	≥1000 Units
Peroxidase	≥10000 Units
Stabilizer and nonreactive ingredients	

Precautions

Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability

Store Catachem's Lactate reagent at 2-8°C. When stored as directed the reagent is stable until expiration date stated on the label. The Catachem Lactate Reagent has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week.

Working Reagent Preparation

Catachem's Lactate reagent is packaged in ready-to-use form. No preparation is required. Once opened, Catachem's Lactate Reagent is stable for 60 days at 2-8°C.

Reagent Indications of Deterioration

- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 550 nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed contact Catachem's technical service.

Specimen Collection and Stability

To maintain sample integrity and avoid changes in Lactate concentrations, care should be taken to collect the sample specimens. Fasting patients should be completely at rest for two hours prior to sampling to allow the Lactate concentrations to reach steady state. Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied. Specimens should be collected in tubes with heparin/fluoride or heparin/iodoacetate as anticoagulants. Separate immediately from the cells and analyze promptly or store at 2-8°C. CSF samples should be collected with addition of glycolysis inhibitor, e.g. sodium fluoride. Lactate in CSF is stable for 3 hours at room temperature, for 24 hours at 2-8°C and 2 months at -20°C.

Procedure

These instructions are outlined for performing the Lactate assay by manual or automated procedures. Read the original instrument manufacturer's instructions and procedures before performing this as an automated Lactate procedure.

Materials Provided

Catachem's Lactate Reagent

Materials required but not Provided

- Analyzer (spectrophotometer) with 550 – 600 nm wavelength
- Calibrator material with assigned lactate value
- Quality control material with assigned lactate values

Calibration

Catachem's Catalac C1200-10 with assigned lactate value is recommended.

Calibration Schedule

Calibration should be performed when this method is run manually or implemented on an automated analyzer for the first time. Recalibration is required after changes of reagent lot number, major instrument service, and when quality control values are out of the indicated range.



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Calibration Procedure

Instructions for calibrating any automated analyzer are provided by the specific instrument manufacturer. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control

To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Catalac (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Directions for Use

Catachem's Lactate method requires one reagent.

Procedure Limitations

Samples with Lactate values greater than 25 mmol/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Interfering Substances

The following substances have no significant effect on the accuracy of this Lactate procedure at the concentrations stated.

- Hemoglobin ≤ 200 mg/dL
- Triglycerides ≤ 1000 mg/dL
- Bilirubin ≤ 2.2 mg/dL

Other substances and certain drugs are also known to influence the lactate values. ⁽⁷⁾

Expected Values - Human

The analytical measuring range of this assay as performed below is 0.5 mmol/L to 2.2 mmol/L. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Analytical Parameters

Wavelength	600nm
Temperature	37° C
Path length	1 cm
Reaction Mode	End Point
Reaction Time	5 minutes
Reaction Volume	0.6 ml
Sample Volume	0.005 ml
Total Volume	0.605 ml
Sample-to-reagent ratio	1:121

Assay Procedure

1. Pour the required volume of Lactate Working Reagent needed dependent on the number of samples to be assayed.
2. Set spectrophotometer temperature at 37°C, wavelength at 600 nm.
3. Pipette 0.6 ml of Lactate reagent into each cuvette marked "Blank", "Test", "Calibrator" and "Control".
4. Pipette 0.005 ml of Blank (water), Control or Test sample into their respective cuvettes.
5. Mix all cuvettes and incubate all cuvettes for 5 minutes.
6. Read absorbance (A) of each cuvette marked "Blank", "Test", "Calibrator" and "Control" at the set wavelength of 600 nm.

Lactate Procedure Scheme				
	RGT BLANK	CAL	CONTRO L	TEST
Lactate Rgt.	0.6 ml	0.6 ml	0.6 ml	0.6 ml
Water (Blank)	0.005 ml	-	-	-
Sample	-	0.005	0.005	0.005
Mix	All cuvettes			
Incubate	All cuvettes at 37°C for 5 minutes			
Calculate	Lactate mmol/L			

Calculations and Results:

$$\text{Lactate (mmol/L)} = \frac{(\text{A}) \text{ Sample} - (\text{A}) \text{ Blank}}{(\text{A}) \text{ Calibrator} - (\text{A}) \text{ Blank}} \times \text{Calibrator (mmol/L)}$$

Example: $\frac{(\text{A}) \text{ Blank}}{0.05} \quad \frac{(\text{A}) \text{ Test}}{0.25} \quad \frac{(\text{A}) \text{ Calibrator}}{0.50}$

Calibrator = 0.5 mmol/L

$$\text{Lactate} = \frac{0.25-0.05}{0.50-0.05} \times 5 = 2.2 \text{ mmol/L}$$

Method Performance Characteristics

Sensitivity: Using a pathlength of 1 cm, a Δ-absorbance of 0.05-0.08 per mmol/L should be obtained.

Linearity: This procedure is linear over the range of 0-25 mmol/L.



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Bibliography

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