LACTATE (2-8°C)

CATALOGUE NUMBER	KIT SIZE (ml)
MPRLAC1	1x25ml / 1x5ml
MPRLAC2	5x20ml / 1x5ml

Intended Use:
For ***In Vitro*** diagnostic use by trained professionals only.
This reagent is intended for the quantitative determination of lactate in human plasma, cerebrospinal fluid.

Clinical Significance:
Anaerobic glycolysis markedly increased blood lactate and causes some increase in pyruvate levels, especially with prolonged exercise. The common cause for increased blood lactate and pyruvate is anoxia resulting from conditions such as shock, pneumonia and congestive heart failure. Lactic acidosis may also occur in renal failure and leukaemia.
Lactate levels in cerebrospinal fluid (CFS) are increased in bacterial meningitis. Increased levels in CFS occur in hypocapnia, hydrocephalus, brain abscesses, cerebral ischemia and generl clinical conditions associated with reduced oxygenation or the brain and/or increased intercranial pressure. This method uses an enzymatic reaction to convert lactate to pyruvate. The hydrogen peroxide produced by this reaction is then used in an enzymatic reaction to generate a coloured dye. This method offers longer reagents stability than the previous UV enzymatic methdos.

Test Principle:
Lactate oxidase converts lactate into pyruvate and hydrogen peroxide, the latter of which reacts in the presence of peroxidase with 4-aminoantipyrine and phenol to a red quinoneimine dye. The increase in colour intensity can be measured spectrophotometrically and is proportional to the lactate concentration in the sample.

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOX}} \text{Pyruvate} + H_2O_2$$
$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{Chromogen} + 4H_2O$$

Reagent Composition

REAGENT	COMPONENT	CONCENTRATION
Lactate Reagent R1	Pipes Buffer pH 7.5	50 mmol/l
	TBHB	6 mmol/l
	LOX	0.2 kU/l
	POD	3 kU/l
	4-aminoantipyrine	0.4 mmol/l
Lactate Standard	Lactate	30 mg/dl (3.33 mmol/l)

Reagent Preparation and Stability:
R1: Liquid, ready to use
Standard: Liquid, ready to use
R1 and Standard are stable until the stated expiry date when stored unopened at 2 - 8°C. Once opened the reagents are stable for a period of 3 weeks, when stored without contamination at 2 - 8°C. **PROTECT FROM LIGHT.** Colouration of the reagent (reagent blank at 546nm, 1cm > 0.2) indicates contamination or damage due to storage at higher temperatures.
On board stability of the reagent is 30 days and of standard is 28 days.
Dispose of reagents carefully in line with local guidelines.

Sample / Sample Preparation / Sample Stability: DO NOT USE SERUM
Use plasma from blood collected by standard venepuncture techniques into fluoride-oxalate tubes (2.5mg sodium fluoride and 2 mg potassium oxalate/ml of blood). Centrifuge within 15 minutes of sample withdrawal. Stable for 2 days at 2 - 8°C and 2 hours at 20 - 25°C .
CSF can be used straight as collected. Stable for 1 day at 2 - 8°C and 3 hours at 20 - 25°C .
Use whole blood collected in sodium fluoride/potassium oxalate tubes. Whole blood needs to be deproteinised by mixing 100ul of whole blood and 200ul of Perchloric acid (0.33N) in a microcentrifuge tube. Vortex the mixture and then centrifuge for 10 minutes at 1500 RCF. Transfer supernatant for analysis. Multiply the whole blood supernatant result by 3 to compensate for dilution. (Note that the whole blood supernatant should be clear to slightly cloudy and colourless).

Note:
1. The lactate level increases rapidly during physical exercise. The time required for return to normal lactate values depends on the physical fitness of the subject. Thirty minutes at rest is usually sufficient for this purpose.
2. Blood samples should be drawn from a stasis-free vein. However, minimal haemostasis (< 30 seconds) will not affect lactate levels. Avoid the use of a tourniquet if possible.
3. Glycolysis in blood samples can rapidly increase the lactate levels. Cells contribute to the glycolysis and their quick removal is essential for the accurate lactate analysis. Heparinized plasma is acceptable, but precautions must be taken to retard glycolysis by keeping the whole blood on ice and then separating the plasma from the cells within 15 minutes of collection.

Assay Procedure:

WAVELENGTH	546nm (492 – 550nm)
TEMPERATURE	25/30/37°C
CUVETTE	1cm Path Length
BLANK	Reagent Blank

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl
Mix and incubate for 5 minutes at 37°C or 10 minutes at 25°C. Read the absorbance (Δ Abs) of Sample/Standard against the Reagent Blank within 30 minutes.			

Calculation:
Concentration (mg/dl) = $\frac{\Delta Abs\ Sample}{\Delta Abs\ Standard}$ x Concentration of Standard

Assay procedure for Deproteinisation methods:

	Blank	Sample
Supernatant	-	50 µl
Distilled Water	50 µl	-
Reagent	1000 µl	1000 µl
Mix and incubate for 5 minutes at 37°C. Read the absorbance (Δ Abs) of Sample/Standard against the Reagent Blank within 30 minutes.		

Performance Characteristics:
Measuring range:
2.0 - 140 mg/dl (0.2 - 15.55 mmol/l)
Dilute samples with higher concentrations using Normal saline 1+1 and rerun the assay. Multiply the result by the dilution factor (for 1+1 dilution, the dilution factor is 2)
Prozone occurs for samples with exceedingly high lactate concentration resulting in Δ Abs within the measuring range. However, these results are flagged in most automated systems to indicate the necessity of a rerun with diluted samples.
Analytical Sensitivity: (Lowest detection limit):
2 mg/dl (0.22 mmol/l)
Imprecision
Intra-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Human Plasma	1.80	0.051	2.83
Level 1	3.35	0.059	1.76
Level 2	3.46	0.074	2.14

Inter-Assay Precision:

Sample	Mean (mg/dl)	SD (mg/dl)	CV %
Human Plasma	1.67	0.028	1.68
Level 1	3.18	0.045	1.37
Level 2	3.39	0.024	0.71

Method Comparison:
AMS Lactate (Y) was compared with another commercially available reagent (X) and gave the following results:
Y = 1.040 x - 0.13 r = 0.997

Interferences:
Criterion: Recovery within +/- 10% of initial value.
Icterus: No significant interference up to 60 mg/dl of Bilirubin
Haemolysis: No significant interference up to 220 mg/dl of Haemoglobin
Lipaemia: No significant interference up to 1000 mg/dl of Triglycerides.

Reference Range:

Sample	Reference Value
Plasma (venous)	4.5 – 19.8 mg/dl (0.5 - 2.2 mmol/l)
CSF Neonate	10 – 60 mg/dl (1.1 - 6.7 mmol/l)
CSF > 10 days old	10 – 25 mg/dl (1.1 - 2.8 mmol/l)
CSF Adult	10 – 22 mg/dl (1.1 - 2.4 mmol/l)
Whole Blood –venous	8.1-15.3 mg/dl (0.9 - 1.7 mmol/l)
Whole Blood - arterial	< 11.3 mg/dl (< 1.3 mmol/l)

Each laboratory should establish its own mean reference range according to the population.
Automated systems:
Contact AMS Diagnostics Technical Department for applications on a wide range of automated analysers.
For automation we recommend the use of a serum based calibrator.
Quality Control and Calibration Material:
Calibration Serum: **QCCCAL1 / QCCCAL2**
Human Assayed Control Normal: **QCCHAN1 / QCCHAN2**
Human Assayed Control Elevated: **QCCHAE1 / QCCHAE2**
Two point calibration is recommended after lot change or as required following quality control procedures.
References:

1. Bablock W et al. A general regression procedure for method transformation. J Clin Chem Biochem. 1988;26:783-790
2. Barhan D, Tinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97. 1972:142
3. Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 2nd ed. Philadephia, PA: W.B. Saunders Co; 1994:976
4. Tietz NW. clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders Co; 1995:382-383
5. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin. Biochem. 1969;6:24
6. Westgard JO, Lahmeyer BL, Birnbaum ML. Clin Chem 1972;18:1334-1338

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		Keep away from sunlight

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