

036-CL10

L-lactic acid Kit

Enzymatic method using difference in pH



V036-CL10-21031

INTENDED USE

036-CL10-L-Lactic acid kit is intended to be used for quantification of L-Lactic acid in whole milk, semi-skimmed milk, skimmed milk, raw milk, pasteurised milk, and UHT milk.

The kit is designed to be used only with the EC CL10 instrument. This kit is not to be used in any human clinical or veterinary diagnostic application.

ASSAY PRINCIPLE

EC Milk L-Lactic Acid is a method for enzymatic determination of L-lactic acid in milk.

As L-lactic acid is oxidized in the reaction catalyzed by lactate oxidase (LOD), H⁺ ions are consumed in the reaction buffer (Enzymatic reaction - see Appendix A).

The variation in pH from the start to the end of the reaction is proportional to the total L-lactic acid concentration in the sample.

KIT COMPONENTS

R1 : Buffer, to be reconstituted – 2 bottle with 150 mL

R2 : NADH/KCl – powder – 2 pcs

R3 : LDH – lactate dehydrogenase – 2 vials with 1.5 mL each

R4 : LOD – Starter, ready to use – 2 vials with 0.8 mL each

R5 : Calibrator – L-lactate 50 mg/L, ready to use – 1 vials with 1.0 mL

EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

For preparation of reagents

Pasteur pipette

For the assay procedure

No. 1 Micropipette Gilson M25 and respective tips

EC CL-10 Plus (Software version 4.3 or higher)

EC Polif Solution (Part No. GEN718)

TEST PROCEDURE

REAGENT PREPARATION

- Important:** allow the reagents to come to room temperature (18-30 °C).
- Do not interchange reagents between kits with different batch numbers.
- Prepare the reagents in the EC Polif Solution kit according to the package insert included with that kit.

Working Buffer (for 25 tests)

- Add 1 mL of **R1 Buffer** into one vial **R2 NADH**.
- Transfer this solution back into the R1 buffer using a Pasteur pipette.
- Rinse vial of R2 NADH twice with aliquots of R1 Buffer, using the same Pasteur pipette.
- Transfer the entire contents of one vial of R3 LDH into the R1 Buffer.
- Rinse R3 LDH with aliquots of R1 Buffer.
- Mix gently.

SAMPLE PREPARATION

Important: Allow the samples to come to room temperature (18-30 °C).

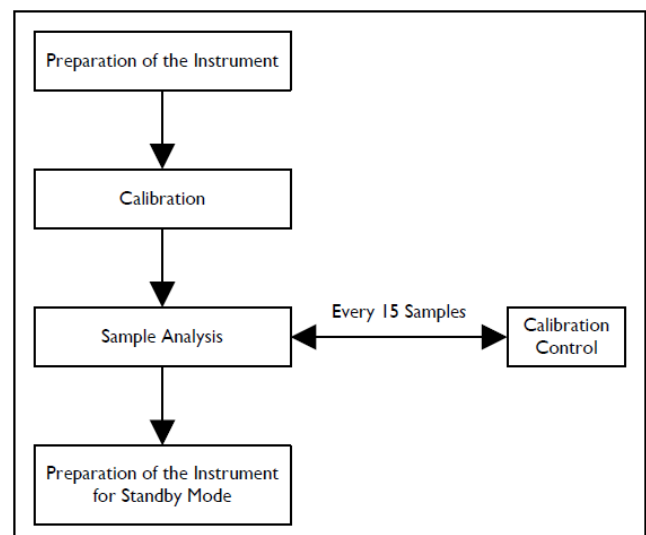
Mix each sample gently prior to the assay.

SAMPLE ANALYSIS

The differential pH measurement is performed in the EC CL-10 Plus instrument.

The procedure is described in detail on page 2-3 of this manual; Figure 1 defines the different steps.

For trouble-shooting and maintenance, please consult the CL-10 Plus Operator Manual.



INSTRUMENT PREPARATION

The results of the assay are given in mg/L. Users who prefer a different unit can modify the setting at this stage, see Appendix C.

The EC CL-10 Plus is normally left on standby (REST) overnight with wash intervals of 90 minutes. If this is not the case consult the instrument operating manual for instructions.

(a) Stop the REST mode or start the EC CL-10 Plus software. In the File menu choose Select method and click LACTIC_MR4.MD.



(b) Replace the EC Polif solution with the reconstituted working buffer:
Wipe the outside of the inlet tube going from buffer pump 4 and insert into the bottle.

(c) Replace the vial containing distilled water with the **R4 LOD**:
Wipe the starter needle going to enzyme pump 6 and insert it the **R4 LOD**.

(d) Empty the waste bottle.



(e) Run Prime enzyme once using the prime enzyme icon or the F2 function key.

(f) Run Clean twice using the clean icon or the F3 function key.

(g) Wait until the system has reached working temperature (37°C). The yellow diode (LED) of the EC CL-10 Plus will flash at 10-20 s intervals.



(h) Run Sample once using the GO icon or the F5 function key. Press Start measure and press Accept. Check that the obtained Δ mpH/min value is between -2 and +4. Take note of the value.

If the result is out of range, Run Sample again*.



(i) Run Sample again using the GO icon or the F5 function key. Check that the obtained Δ mpH/min value is between -2 and +4 and it is not different from the point 8 value more than ± 0.7 mpH.

If the result is out of range, run Sample again*.

CALIBRATION

(a) Check if the system is at working temperature (37°C). The yellow diode (LED) will flash at 10-20s intervals.



(b) Run *Blank* once using the blank icon or the F6 function key. Check the *Offset* values: [Min: -2, Max: 4].

If the result is displayed in green (upper window on the right), proceed to step E. (c).

If result is displayed in red repeat from step E. (a)*.

(c) Using the micropipette Gilson M25 dispense 2 x 20 μ L of **R5 Calibrator** into the mixing chamber. Pipetting instructions can be provided on request by your local Biosentec representative.



(d) Run *Calibrate* once using the calibrate icon or F7 function key.

Check the *Slope* values: [Min: 2.1, Max: 5.0]. If the result is correct the value will be 50 and it will be displayed in green. If this is the case, proceed to step E (e).

If the result is displayed in red (out of range), repeat from step E. (a)*.

(e) Using a Gilson M25 micropipette dispense 2 x 20 μ L of R5 Calibrator into the mixing chamber.



(f) Run Sample once using the GO icon or the F5 Function key. Press Start measure, then type "check cal" in sample id and press Accept. The result must be equal to the STD value $\pm 6\%$ (see Appendix B).

If the result is in range, proceed to step F. (a).

If the result is out of range, repeat step E. (e) and run Sample again.

(g) If the result is in range, repeat step 14 and run Sample again. If the result is in range this second time, proceed to step 16. If the result is out of range, repeat from step E. (a)*.

* If the result is still out of range, please contact Biosentec for further assistance.

SAMPLE ANALYSIS

Note: It is important that the sample is at room temperature and homogeneous when analyzed.

Perform the measurements in a sequence. If you wait more than five minutes between two consecutive tests, enter GO without injecting any sample.



- (a) Using the micropipette Gilson M25 dispense 2 x 20 μ L of the sample into the mixing chamber.
- (b) Run Sample using the GO icon or the F5 function key.
Press Start measure and, if needed, type in the sample identification and press Accept.
The results can be read from the screen or printed out.

CONTROL OF THE CALIBRATION OF THE INSTRUMENT

Note: A control of the instrument calibration is needed after 15 samples.

Repeat steps E. (e) and E. (f) of the calibration procedure.

PREPARATION OF THE INSTRUMENT FOR STANDBY MODE

- (a) At the end of the working session place the left-over reagents in the refrigerator at 2-8 °C.
- (b) Replace the reconstituted working buffer with the reconstituted wash solution from the EC Polif Solution kit.
- (c) Insert the starter needle going into enzyme pump 6 into a vial containing at least 2 mL of distilled water.
- (d) Empty the waste bottle
- (e) Run Prime enzyme once using the prime enzyme icon or the F2 function key.
- (f) Run Clean twice using the clean icon or the F3 function key.
- (g) Check that enough diluted wash solution is left for the estimated standby period (the wash cycle automatically runs every 90 minutes and consumes about 4.4 mL of reconstituted wash buffer for each cycle).
- (h) In the Service menu, choose Enter REST mode.
- (i) Leave the instrument with the power on. Turn the monitor off.



Note: If the instrument will not be used for a longer period of time make sure that a sufficient amount of wash buffer is available or consult the operating manual for instructions on shutting down the instrument

STORAGE CONDITIONS

The kit components must be stored at 2-8 °C.

The reconstituted working buffer is stable for one week at 2-8 °C.

SAFETY

Good laboratory practice should be employed when using this kit. Safety clothing should be worn and skin contact with reagents avoided. Do not ingest.

Material safety data sheets are available on request.

PERFORMANCE CHARACTERISTICS

Limit of detection

The limit of detection is 0.055 mM (5 mg L-lactic acid/L)

Linearity

The performance characteristics are valid within the range 0.055-0.89 mM (5-80 mg/L)

Precision

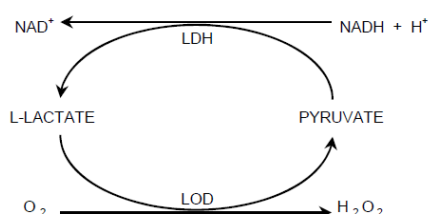
The repeatability of the results is within the range +/- 0.033 mM (+/- 3 mg/L)

Accuracy

The variation compared with the reference method is within the range +/- 0.044 mM (+/-4 mg/L)

APPENDIX A – ENZYMATIC REACTION

The enzymatic reaction taking place in the reaction chamber are described as:



APPENDIX B - CONVERSION TABLE

	L-lactic acid mg/L	L-Lactic acid mM
STD value	50	0.555
STD min	47	0.522
STD max	53	0.588
Cnv factor	1	0.011

APPENDIX C – INSTRUMENT SETTINGS

If you wish to modify the unit of the results or to visualize the settings of the method to be used: from the File menu, go to Edit method and select the Lactose method (LACTIC_MR4.MD). See Figure 2.

If you wish modify the units of the result; insert the desired unit in the field User units and the conversion factor (see Appendix B) in the field Cnv factor. Click on “Save”.

Your settings will be saved as LACTIC AC. (see Short Name field). Rename your file: enter Edit method and delete LACTIC_MR4.MD; rename the new file LACTIC AC. as LACTIC_MR4.MD.

Note: Do not change the other parameters in this window without consulting your Biosentec representative.

Figure 2 – Instrument settings

The screenshot shows the instrument settings window for LACTIC AC. The window is divided into several sections:

- Method definition:** Short Name: LACTIC AC., Description: L-Lactate, Execution: Kinetics, Calc Type: None, STD units: mg/l, User units: , Cnv factor: 1.000+E
- Offset Parameters:** Offset value: 0.000, Minimum offset: -2.000, Maximum offset: 4.000
- Standard:** Standard Value: 50.000
- Results:** Min. Result: 0.0, Max. Result: 80.0, Dec. Digits: 1
- Quality Control:** Control Value: 0.00, Std Dev: 1.00
- Execution parameters:** Temperature (°C): 37.0, Measure Time (sec.): 45, Wait Time (sec.): 2, Lag Time (sec.): 15
- Slope Parameters:** Slope value: 3.3000, Minimum slope: 2.1000, Maximum slope: 5.0000
- Instrument initialization string:** CS1000_

At the bottom, there are navigation buttons: Prev, 1 of 1, Next, Add Method, Save, and Cancel.

EXP use before
Date d'expiration

REF catalogue number
N° dans le catalogue



Attention



Biosentec
48 chemin des Palanques Sud
31120 Portet sur Garonne

LOT Lot
N° de lot



Store at 2-8°C
Conserver à 2-8°C



Notice utilisation
Operation note