035-CL10

Glucose/Lactose Kit

Enzymatic method using difference in pH

Reference method according to ISO 26462:2010 / IDF 214:2010

BioSenTec

V035-CL10-21031

INTENDED USE

035-CL10-Glucose/Lactose kit is intended to be used for quantification of lactose in raw milk, pasteurised milk, UHT milk and powdered 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 Lactose is a method for enzymatic determination of lactose in milk.

Lactose is a disaccharide, made of one molecule of glucose and one molecule of galactose.

After completion of the reaction lactose is cleaved into glucose and galactose in the reaction catalyzed by β-galactosidase. As soon as glucose is generated, it is processed by the hexokinase (HK) enzyme. H+ ions are produced in the reaction buffer (Enzymatic reaction – see Appendix A). The variation of pH from the start to the end of the reaction is proportional to the total lactose concentration in the sample.

Glucose free in sample is directly eliminated by the reaction because sample and enzyme (HK) are present in both electrodes.

KIT COMPONENTS

R1 : Concentrated buffer, to be reconstitued
1 bottle with 45 mL

R2 : Concentrated diluent – glucose/lactose diluent, to be reconstituted – 1 bottle with 45 mL

R3 : Hexokinase starter, ready to use
2 vials with 0.8 mL each

R4 : β-Galactosidase starter, ready to use
2 vials with 0.9 mL each

R5 : Calibrator – Glucose 50 mM + Lactose 100 mM, ready to use – 2 vials with I.0 mL each

EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

For preparation of reagents

25-50 mL measuring cylinder
250 mL bottle (i.e. Kartell bottle)
Distilled water

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

(a) **Important**: allow the reagents to come to room temperature (18-30 °C).

(b) Do not interchange reagents between kits with different batch numbers.

(c) Prepare the reagents in the EC Polif Solution kit according to the package insert included with that kit.

Working Buffer (for 25 tests)

1. Measure 160 mL of distilled water into a bottle (i.e. a 250 mL Kartell bottle).
2. Add 20 mL of R1 Concentrated Buffer (vial 1).
3. Add 20 mL of R2 Concentrated Diluent (vial 2).
4. Mix gently.

SAMPLE PREPARATION

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

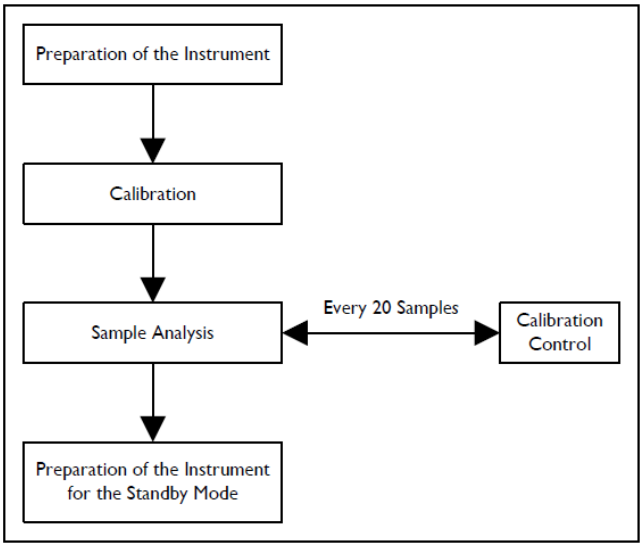
Mix each sample gently prior to the assay.

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 mM. 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.

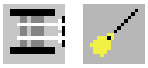
(a) Stop the REST mode or start the EC CL-10 Plus software. In the File menu choose Select method and click LACTOSE_MR1.MD.

(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 β-Galactosidase:
Wipe the starter needle going to enzyme pump 6 and insert it the vial of β-Galactosidase.

(d) Place R3 Hexokinase in position next to the vial of R4 β-Galactosidase on the instrument.

(e) Empty the waste bottle.



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

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

(h) 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.

(i) Using the micropipette Gilson M25 inject 20 µL of the R3 Hexokinase into the mixing chamber.

(j) Run Sample once using the GO icon or the F5 function key. Press Start measure and press Accept. Check that the obtained ΔmpH value is between –20 and +5. Take note of the value.

If the result is out of range, inject 20 µL of R3 Hexokinase into the mixing chamber and run Sample. Press Start measure and press Accept.

(k) Repeat steps D. (i) and D. (j). Check that the obtained ΔmpH value is not different from the value noted in step D. (j) more than ± 1.0 mpH. If it is, repeat steps D. (i) and D. (j) once more.*

CALIBRATION

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

(b) Using the micropipette Gilson M25 inject 20 µL of **R3 Hexokinase** into the mixing chamber.

(c) Run *Blank* once using the blank icon or the F6 function key.
Check the *Offset* values: [Min: -20, Max: 5].

If the result is displayed in green (upper window on the right), proceed to step E. (d).
If result is displayed in red repeat from step E. (a)*.

(d) Using the micropipette Gilson M25 dispense 20 µL of **R3 Hexokinase** and with the same micropipette dispense 20 µL of **R5 Calibrator** into the mixing chamber.

(e) Run *Calibrate* once using the calibrate icon or F7 function key.
Check the *Slope* values: [Min: -1, Max:-0.5].

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

(f) Using the micropipette Gilson M25 dispense 20 µL of **R3 Hexokinase** and with the same micropipette dispense 20 µL of **R5 Calibrator** into the mixing chamber

Run *Sample* using the GO icon or the F5 function key. Press *Start measure*, and then type "check cal" in sample id and press *Accept*. The result must be equal to the STD value ±2 % (see Appendix B).

(g) If the result is in range, proceed to step F. (a).
If the result is out of range, repeat step E. (f) and step E. (g).
If the result is in range, repeat step E. (f) and step E. (g).
If the result is in range this second time, proceed to step F. (a).
If the result is out of range, repeat from step E. (a).
*If the result is still out of range, contact your Biosentec representative for 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 20 µL of R3 Hexokinase and 20 µL 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 15-20 samples.

Repeat steps E. (f) and E. (g) 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.

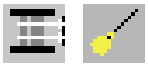
(b) Replace the reconstituted working buffer with the reconstituted wash solution from the EC Polif Solution kit.

(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 4.0 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

PERFORMANCE CHARASTERISTICS

Limit of detection

The limit of detection is 3 mM Lactose (1.1 g/L)

Linearity

The performance characteristics are valid within the range

Lactose 3-200 mM 1.03-68 g/L lactose anhydrous
1.1-72 g/L lactose monohydrate

Precision

The repeatability of the results is within the range +/- 1.5 mM at 100 mM (+/- 0.54 g/L at 36 g/L)

Accuracy

The variation compared with the reference method is within the range +/- 1.5 mM at 140 mM (+/-0.7 g/L at 50 g/L)

APPENDIX A – ENZYMATIC REACTION

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

lactose + ATP + HK ⟶ glucose-6P + ADP + H^+ + galactose

APPENDIX B - CONVERSION TABLE

Lactose	mM	Anhydrous g/100 gr	Anhydrous g/100 ml	Monohydr g/100 gr	Monohydr g/100 ml
STD value	100	3.32	3.42	3.49	3.60
STD min	98	3.25	3.35	3.43	3.53
STD max	102	3.39	3.49	3.57	3.67
Cnv factor	1	0.03320	0.03420	0.03498	0.03603

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 (LACTOSE_MR1.MD). See Figure C1.

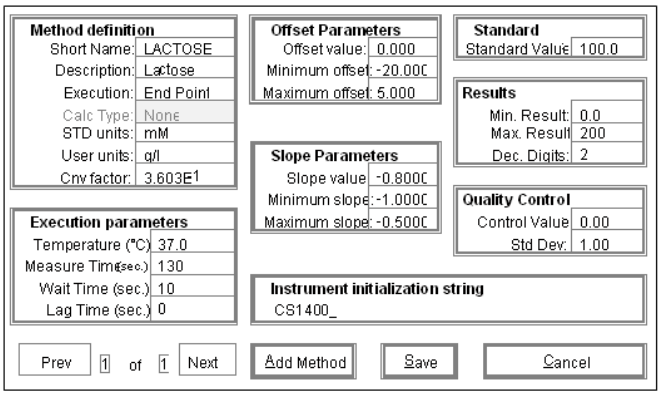
If the version number of the method file (*.MD) does not correspond to the one in this package insert, then contact your Biosentec representative.

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 LACTOSE (see Short Name field). Rename your file: enter Edit method and delete LACTOSE_MR1.MD; rename the new file LACTOSE as LACTOSE_MR1.MD.

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

Figure C1 – Instrument settings



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*	2°C 8°C	*Store at 2-8°C* *Conserver à 2-8°C*		*Notice utilisation* *Operation note*		