

# Glucose/Lactose Kit

Enzymatic method using difference in pH

Reference method according to ISO 14637:2004 / FIL 195:2004

## INTENDED USE

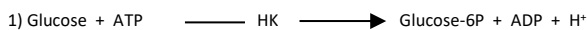
035-EFA-Glucose/Lactose kit is intended to be used for the quantification, in a single test via two coupled reactions, of both glucose and lactose in raw and pasteurized milk.

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

## ASSAY PRINCIPLE

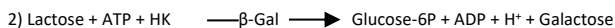
Lactose is a disaccharide formed by one molecule of glucose and one molecule of galactose linked by a  $\beta$ -glucoside bond; it can be hydrolyzed by the specific enzyme  $\beta$ -galactosidase.

The assay is carried out according to the following principle:



After sample injection, the addition of hexokinase (HK) enzyme will start reaction 1. The enzyme will process all the free glucose present in the sample.

The variation of  $\text{H}^+$  is proportional to the amount of free glucose in the sample.



After completion of reaction 1, the addition of  $\beta$ -galactosidase promotes hydrolysis of lactose and triggers reaction 2. As soon as glucose is generated by hydrolysis, it is processed by the HK still present in the reaction mixture.

The variation of  $\text{H}^+$  is proportional to the amount of lactose in the sample.

## KIT COMPONENTS

**R1** : Phosphate/TRIS buffer pH 7.9, stabilizers  
1 bottle with 12 mL

**R2** : Concentrated diluent – KCl, Mg Cl<sub>2</sub>, detergents  
1 bottle with 40 mL

**R3** : Hexokinase (HK) – 300 U/mL, stabilizers  
2 vials with 1.1 mL each

**R4** :  $\beta$ -Galactosidase ( $\beta$ -Gal) – 2000 U/mL, stabilizers  
2 vials with 1.1 mL each

**R5** : Calibrator – Glucose 50 mM + Lactose 100 mM, stabilizers  
2 vials with 1.5 mL each

## REAGENT PREPARATION

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

R2 Concentrated Diluent has to be diluted 1 to 9 with distilled water and connected with peripump no. 3.

Reagents 1, 3, 4 and 5 are ready to use.

## SAMPLE PREPARATION

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

In order to get accurate results all samples should be homogenized before testing. If particles larger than 0.3mm are present, filtration or centrifugation is recommended to get rid of them.

Dispense 0.5 mL minimum sample volume in the Microlab® EFA cups in order to prevent fat, which floats on the surface of the liquid, from affecting the assay results. Fat can stick to the dispensing needle and not be properly washed away. Whenever this happens sample may be underestimated.

## TEST PROCEDURE

EC Milk Glucose/Lactose kit is a specific method to be used on the EC Microlab EFA instrument and is included in the EFA Instrument Software (regarding the use of this software please consult the EC Microlab EFA User Manual).

Positioning on deck:

R1	Buffer rack
R2	+ dist.water To be connected to peripump n. 3
R3	Enzyme rack
R4	Enzyme rack
R5	Calibrator rack

Click on the *Edit Test Settings* button and make sure that all parameters for *GlucoseLactose* given in the tables below are set correctly.

Prime needle and electrodes (*BeginWork*).

Start the method by clicking the *GlucoseLactose* method execution button.

## TEST SETTINGS

### Multiple tests

First parameter

EnzymePos1	1
------------	---

Second parameter

EnzymePos2	2
------------	---

### Volumes and Positions

Reagent Positions

The positions BufferPos (R1), CalibPos (R5) and SampleStart are freely defined by user (default is 1 for R1 and R5). The positions EnzymePos1 (R3- default pos.:1); EnzymePos2 (R4, - default pos.:2) outlined in the first and second

parameter folders of the Multiple Test settings, are also freely defined by the user. Make sure that all positions match the current state of the deck. Reconstituted R2 must be placed on the working area and connected to the buffer pump 3.

Specials

Number of Jobs

Blank	2	ControlSet	0
Calibs	1	ControlFreq	0
CalibCheck	1	Replicas	1
CalibFreq	100	PreLoad	1

The number of repetitions can be freely defined by user (default is 1); this refers to the number of duplications for each sample.

CALCULATION

The reaction is linear and the calculation is automatically done by software according to the following equations:

$$\text{Slope} = \text{Calibrator Concentration} / \text{Calibrator Signal}$$

$$\text{Sample Concentration} = \text{Slope} \times \text{Sample Signal}$$

UNITS

The concentration of the calibrator in the kit is expressed in mM. Units may be changed only for samples by editing the conversion factor and Sample User Units in the Excel sheet.

This operation must be carried out before starting a method. In the EFA Instrument application select *Milk book* and press the button *Go to Excel book*. On Excel edit the *Sample User Units* and the conversion factor and save the file.

Example: If the desired unit is g/L instead of mM, edit g/L in the cell adjacent to the *Sample User Units* cell and enter 0.18 in the cell adjacent to *Conversion Factor* cell.

NOTE: do not edit the other cells!

Conversion Factor	User Units
1	mM
0.3603	g/L lactose monohydrate
0.342	g/L lactose anhydrous
0.03603	g/100mL lactose monohydrate
0.0342	g/100mL lactose anhydrous

SPECIFICITY

The reaction is specific for both glucose and lactose.

TEST SPECIFICATIONS

*Glucose*

Linearity: 5 - 200 mM (1 - 36 g/L)

Precision : ±2 mM at 50 mM (±0.4 g/L at 9g/L)

*Lactose*

Linearity: 5 - 200mM (1.7 - 68g/L lactose anhydrous)  
(1.8 – 68g/L lactose monohydrate)

Precision : ±2 mM at 100 mM (±0.7 g/L at 36g/L)

NO. OF TESTS RATIONALE

The composition of the present 035-EFA Glucose/lactose kit is calculated for 3 series with 17 samples, 1 preload, 2 blanks, 1 calibration, 1 check each. Consider that the number of determinations for this test goes below 50 if the number of series will be more than 3.








STORAGE CONDITIONS

The kit components must be stored 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.

 <i>use before</i> <i>Date d'expiration</i>	 <i>catalogue number</i> <i>N° dans le catalogue</i>	 <i>Attention</i>	 <i>Biosentec</i> <i>65 Allée Campferran</i> <i>31320 Auzeville-Tolosane</i>
 <i>Lot</i> <i>N° de lot</i>	 <i>Store at 2-8°C</i> <i>Conserver à 2-8°C</i>	 <i>Notice utilisation</i> <i>Operation note</i>	