034-CL10

Urea Kit

Enzymatic method using difference in pH

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

V034-CL10-21031

INTENDED USE

034-CL10 urea kit is intended to be used for the quantification of urea in whole milk, semi-skimmed milk, skimmed milk, raw milk, UHT and pasteurized milk. No interferences have been detected in samples stabilized with the preservatives Bronopol (broad spectrum microtabs, liquid solution or other tablets) or sodium azide (tablets or liquid solution).

The kit is designed to be used only with the EC *CL-10 Plus* instrument.

ASSAY PRINCIPLE

034-CL10 urea kit is a method for enzymatic determination of urea in milk.

As urea is hydrolyzed in the reaction catalyzed by urease, OH^- ions are produced 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 urea concentration in the sample.

MATERIALS

Kit components

R1 : Phosphate buffer pH 6.7, stabilizers
2 bottles with 250 mL each
Stable at 2-8 °c until expiry date
R2 : Urease, stabilizers
2 vials with 1.8 mL each
Stable at 2-8 °c until expiry date
CAL : Urea calibrator, 100 mg/dL, stabilizers
2 vials with I.8 mL each
Stable at 2-8 °c until expiry date

Equipment and material required but not provided

For the assay procedure

- Micropipette Gilson M25 or equivalent, and respective tips
- EC *CL-10 Plus*
- *Polif Solution* (ref. 034a-Polif Solution)
- Distilled water

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.

TEST PROCEDURE

Preparation of reagents

Important: allow the reagents to come to room temperature (18-30 ^{0}C).

Do not interchange reagents between kits with different batch numbers.

All reagents are ready to use.

Preparation of samples

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

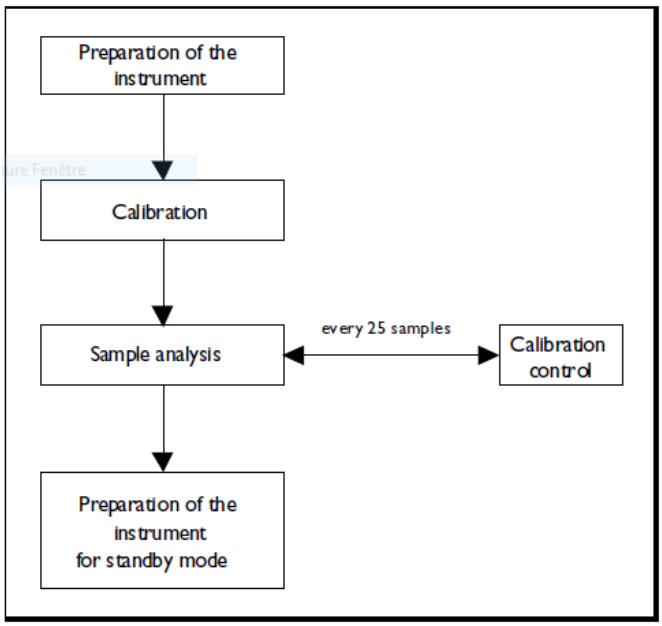
Mix each sample gently prior to assay.

ASSAY PROCEDURE

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

The procedure is described on pages 2-3 of this manual; figure 1 defines the different steps.

For trouble-shooting and maintenance of the EC *CL-10 Plus* please consult the instrument operating manual.



Preparation of the instrument

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

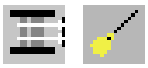
The EC *CL-10 Plus* is normally left on standby (*REST*) overnight with wash intervals of 90 minutes. If this is not the case consult the instrument operating manual for instructions.

1. Stop the *REST* mode or start the EC *CL-10 Plus* software. In the *File* menu choose *Select method* and click on UREA_FIL_R4.MD.

2. Replace the 34a- *Polif* solution with the R1 Buffer (vial 1):
   Connect vial 1 to the peristaltic tube going to buffer pump 4. Wipe the outside of the inlet tube.

3. Replace the vial containing distilled water with the R2 Urease Starter (vial 2):
   Wipe the starter needle going to enzyme pump 6 and insert it into vial 2.

4. Empty the waste bottle.



5. Run *Prime enzyme* once using the prime enzyme icon or the F2 function key.

6. Run *Clean* twice using the clean icon or the F3 function key.

7. 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.

8. In the *Start Up* menu choose *Start up parameters* and check the values:
   Number of cycles: 3, Time Interval: 15 and Max Error: 1. Change the existing values if they are incorrect; validate by clicking on OK.

9. Run *Run Start Up Procedure* once using the start up icon or F4 function key.
   If the result is out of range, a message will appear. If this happens, run *Run Start Up Procedure* again.

Calibration

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

11. Run *Blank* once using the blank icon or the F6 function key.
    Check the *Offset value*: [Min: -3, Max: 3].
    If the result is displayed in green (upper window on the right), proceed to step 12.
    If the result is displayed in red (out of range), repeat from step 10.

12. Using a Gilson M25 micropipette or equivalent, dispense 25 µL of the Urea Calibrator (vial 3 CAL) into the mixing chamber.

13. Run *Calibrate* once using the calibrate icon or the F7 function key.
    Check the *Slope value*: [Min: 2.3, Max: 3.3]. If the result is correct, the value will be 100 and it will be displayed in green. If this is the case, proceed to step 14.
    If the result is displayed in red (out of range), repeat from step 10.

14. Using a Gilson M25 micropipette or equivalent, dispense 25 µL of the R3 Calibrator (vial 3 CAL) into the mixing chamber.

15. 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 ±3% (see Appendix B).
    If the result is in range, proceed to step 16.
    If the result is out of range, repeat step 14 and run *Sample* again.
    - 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 range, repeat from step 10.

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.

16. Using a Gilson M25 micropipette or equivalent, dispense 25 µL of the sample into the mixing chamber.

17. Run *Sample* once 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.

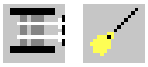
Calibration control

Note: The instrument calibration needs to be checked after analysing 20-30 samples in sequence.

Repeat steps 14 and 15 of the Calibration Procedure.

Preparation of the instrument for standby mode

18. At the end of the session place the left-over reagents in the refrigerator at 2-8 °C.
19. Replace the R1 Buffer (vial 1) with the reconstituted wash solution, from the 034a- *Polif* kit
20. Insert the starter needle going into enzyme pump 6 into a vial containing at least 2 mL of distilled water.



21. Empty the waste bottle.
22. Run *Prime enzyme* twice using the prime enzyme icon or the F2 function key.
23. Run *Clean* twice using the clean icon or the F3 function key.
24. 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).
25. In the *Service menu,* choose *Enter REST Mode*.
26. 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 mg urea / dl sample.

Linearity

The performance characteristics are valid within the range 3-400 mg/dL.

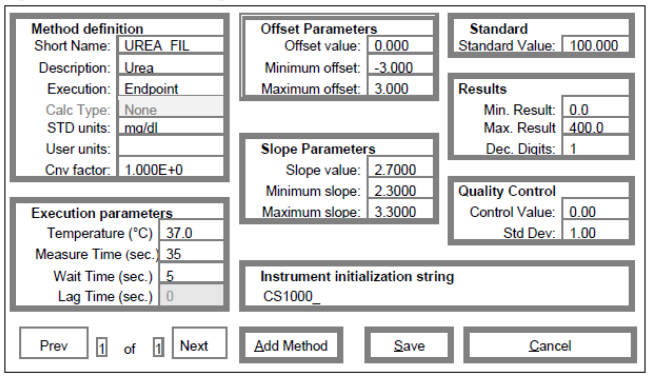
Precision

The repeatability of the results is within the range +/- 1.5 mg/dL.

Accuracy

The variation compared with the reference method is within the range +/- 1.5 mg/dL.

Figure C1 - Instrument settings



Example User unit: MUN (Milk Urea Nitrogen) Cnv factor: 0.4667

Appendix A – Enzymatic reaction

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

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO2$$

$$2NH_3 + 2\,H_2O \longleftrightarrow 2NH_4^+ + 2OH^-$$

$$CO2 + H2O \longleftrightarrow HCO_3^- + H^+$$

Appendix B - Conversion Table

	Urea (mg/dL)	Urea (mM)	Urea (% p/v)	M.U.N (mg/dL)
STD value	100	16.66	0.100	46.67
STD min	97	16.16	0.097	45.27
STD max	103	17.16	0.103	48.07
Cnv factor	1	0.1666	0.001	0.4667

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* then select Urea method (UREA_FIL_R4.MD). See Figure C1.

If you wish to modify the units of the result: insert the desired unit in the *User units* field and the conversion factor (see Appendix B) in the *Cnv factor* field. Click on "Save".

Your settings will be saved as UREA_FIL *(see Short Name* field). Rename your file: enter *Edit method* and delete UREA_FIL_R4.MD file; rename the new file UREA_FIL as UREA_FIL_R4.MD.

Note: *Do not change the other parameters in this window*

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*		