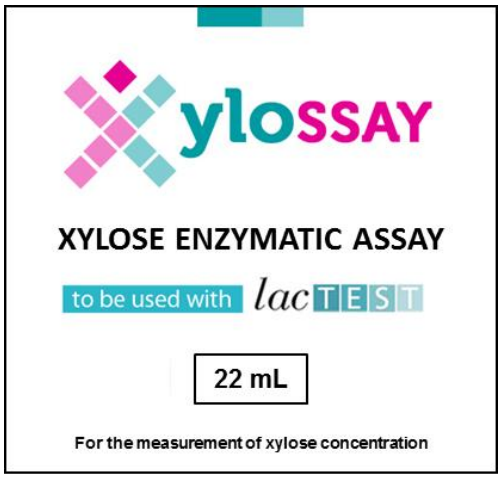
VERSION FOR ROCHE COBAS c501/c502 AUTOMATED ANALYZERS



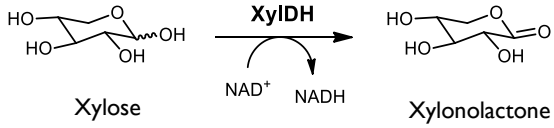
IVD CE

DESCRIPTION:

Xylossay® is a reliable and automatable method for the quantification of xylose present in urine. **Xylossay®** is the recommended method for xylose detection after the administration of **LacTEST 0.45 g.**

METHOD RATIONALE:

The enzyme xylose dehydrogenase (XylDH)[1] catalyzes the specific oxidation of xylose to yield xylonolactone[2]. As XylDH is a NAD^+ dependent enzyme, this reaction requires a concomitant reduction of this cofactor to NADH:



Xylose Xylonolactone

Production of NADH can be spectrophotometrically detected and quantified by the increase of the absorbance at 340 nm during the reaction. This way, absorbance increments will be directly proportional to the amount of xylose present in the sample.

COMPONENTS OF THE KIT:

Xylossay® is supplied in a format suitable for performing 70 xylose determinations. Each kit contains a vial of each one of the following components:

VIAL	CONTENT AND FORMAT	AMOUNT
Brown	Phosphate buffer, 50 mM, pH 8.0 (Solution)	22 mL
Orange	β-Nicotinamide Adenine Dinucleotide (NAD^+) (Lyophilized)	66.34 mg ± 12%
Purple	D-Xylose Dehydrogenase (Lyophilized)	0.6 mg ± 10%
Pink	Calibrator: D-(+)-Xylose (Solution)	2 mL; 3.75 mg/dL ± 5%.

1. Sánchez-Moreno, I. *et al. J. Biotechnol.* (2016) 234:50-57.
2. Stephens, C. *et al. J. Bacteriol.* (2007) 189:2181-2185.

NON SUPPLIED ADDITIONAL EQUIPMENT:

Preparation of the reagents requires the use of micropipettes or systems suitable for dispensing the specified volumes. Vortex agitators can be used when indicated. Xylose quantification controls.

PREPARATION OF REAGENTS:

IMPORTANT: the preparation of the reagents must be conducted in the following order:

1. Dissolve the content of the purple vial (Xylose dehydrogenase, lyophilized) in ***5 mL*** of buffer (Phosphate buffer 50 mM, pH 8.0; brown vial). Mix genteelly to avoid loss of activity in the suspended enzyme. Keep cold during use if possible. This solution will be called **REACTIVE 2**.

2. Add 2 mL of buffer (Phosphate buffer 50 mM, pH 8.0; brown vial) in the vial that contains the lyophilized NAD^+ (orange vial) and mix vigorously until its complete dissolution (vortex agitators can be used).

3. Add the totality of the 2 mL of dissolved NAD^+ (orange vial + buffer) in the buffer vial (brown vial). Thus, the brown vial will contain ***17 mL*** of phosphate buffer plus NAD^+ at the appropriated concentration. Keep cold if possible. This solution will be called **REACTIVE 1**.

4. The solution of xylose (Calibrator: standard xylose solution, pink vial) is ready for use.

CONSERVATION AND STABILITY:

Xylossay® must be stored at temperatures between 4° and 8°C until use, being stable under these conditions for at least 18 months. After being prepared, REACTIVES 1 and 2 are stable for at least 39 days when maintained at temperatures between 4 and 10°C (stability in automated equipments). Both REACTIVE 1 and REACTIVE 2 can be frozen at -20°C without any activity loss. Both reagents can be frozen and thawed at least 6 times without altering their function.

IMPORTANT: to use frozen reactives, these must be completely thawed (at room temperature). Once completely thawed, they must be agitated for total homogenization before use.

ASSAY PROTOCOL:

SAMPLE PREPARATION: Collection of urine samples must be performed strictly following the specifications indicated in **LacTEST 0.45 g** summary of product characteristics. Centrifugation of urine samples is recommended (according to the protocol established in each laboratory) to remove any possible precipitate that could affect the assay. Frozen samples must be agitated after thawing for their homogenization.

PARAMETERS: wavelength 340 nm, using the standard device temperature (usually 37°C). The instrumental application for testing must be designed according to the following specifications.

CALIBRATION AND CONTROL PROCEDURES: Calibration of xylose determination in the analyzers (**CAL**) must be carried out using the standard xylose solution supplied with the kit (Calibrator, pink vial) as concentration reference (3.75 mg/dL). For calibration and xylose quantification control it is recommended the use of at least two certified and validated levels of control: a low level, with xylose concentration between 0.8 and 3 mg/dL, and a high level,

with xylose concentration between 5 and 8 mg/dL. These controls are not supplied with the kit, and must be purchased independently.

STANDARD ASSAY PROTOCOL:

The shown volumes can be increased or reduced if the final proportion between them is maintained.

Reactive	Blank	Sample	CAL
Distilled water	**30 µL**	-	-
REACTIVE 1	**130 µL**	**130 µL**	**130 µL**
Sample	-	**30 µL**	-
Calibrator	-	-	**30 µL**
Mix the reaction and incubate for 5 min. (**A1**)			
REACTIVE 2	**40 µL**	**40 µL**	**40 µL**
Mix the reaction and incubate for 5 min. (**A2**)			

FINAL NUMBER OF MEASUREMENTS WITH EACH KIT:

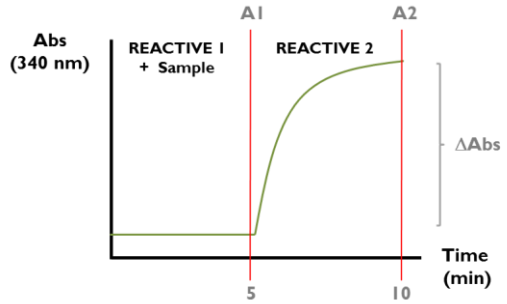
Due to the dead volume of some automated analyzers, the number of determinations can be reduced. The next formulas allow calculating the final number of assays in function of the dead volume:

$$\boldsymbol{REACTIVE\ 1} = \frac{(\mathbf{17\ mL} - \text{Dead volume in mL}) \text{ x } 1000}{\text{Volume of REACTIVE 1 in the assay (µL)}}$$

$$\boldsymbol{REACTIVE\ 2} = \frac{(\mathbf{5\ mL} - \text{Dead volume in mL}) \text{ x } 1000}{\text{Volume of REACTIVE 2 in the assay (µL)}}$$

CALCULATIONS:

Absorbance values (340 nm) per assay:



A1 = Initial absorbance of the mixture REACTIVE 1 + Sample (incubated for 5 min).

A2 = Final absorbance after adding REACTIVE 2 (incubated for 5 more minutes, total time of reaction: 10 min).

Differences between the two absorbance values will be proportional to xylose concentration, which can be calculated using the standard xylose solution provided with the kit (Xylose standard solution, pink vial):

ΔAbsorbance (340 nm) = ΔAbs = A2 - A1

Sample concentration = [*Sample*] (*mg/dL*)

Xylose concentration (Calibrator) = *3.75 mg/dL*

$$[Sample] = \frac{\Delta Abs\ (Sample)}{\Delta Abs\ (Calibrator)}\ x\ 3.75\ mg/dL$$

The total amount of xylose in the Sample (mg) can be calculated using the total volume of urine collected during the test.

$$Xylose\ (mg) = [Sample]\ x\ Volume\ of\ Sample\ (dL)$$

EXAMPLE:

Determination of the total amount of xylose in the urine sample of a patient:

Volume of Sample (urine) = **557 mL** = 5.57 dL

- **Sample assay**: A1 = 0.122

A2 = 0.146

$$\Delta Abs\ (Sample) = 0.146 - 0.122 = 0.024$$

- **Calibrator assay**: A1 = 0.0870

A2 = 0.166

$$\Delta Abs\ (Calibrator) = 0.166 - 0.0870 = 0.079$$

$$[Sample] = \frac{0.024}{0.079}\ x\ 3.75\ mg/dL = 1.139\ mg/dL$$

$$Xylose\ (mg) = 1.139\ \frac{mg}{dL}\ x\ 5.57 dL = \mathbf{6.34\ mg}$$

REFERENCE VALUES

Normal values in adults:

Test	Xylose in urine (mg)
LacTEST 0.45 g	≥ 19.18

Values below 19.18 mg indicate hypolactasia.

ANALYTICAL PARAMETERS:

The analytical parameters of **Xylossay®** application were determined during the validation of the technique in three automated analyzers (Roche Cobas c502, Werfen ILab 600 and Siemens Dimension Vista 1500 analyzers).

Linearity: until at least 15 mg/dL in all the cases.

Limit of detection: between 0.13 and 0.49 mg/dL.

Reproducibility: The coefficients of variation between samples cannot be higher than 15%.

Accuracy: ≥ 88 %.

Carry-over: ≤ 4 %

ANALYTICAL PERFORMANCE:

The analytical parameters established for **Xylossay®** can slightly vary in function of the characteristics of the equipment used. Presence of some sugars as L-arabinose or glucose at high concentrations can affect xylose determination. For this reason, sample collection requires a fasting period of 10 hours. Under these conditions, no interference has been found in the collected urine.

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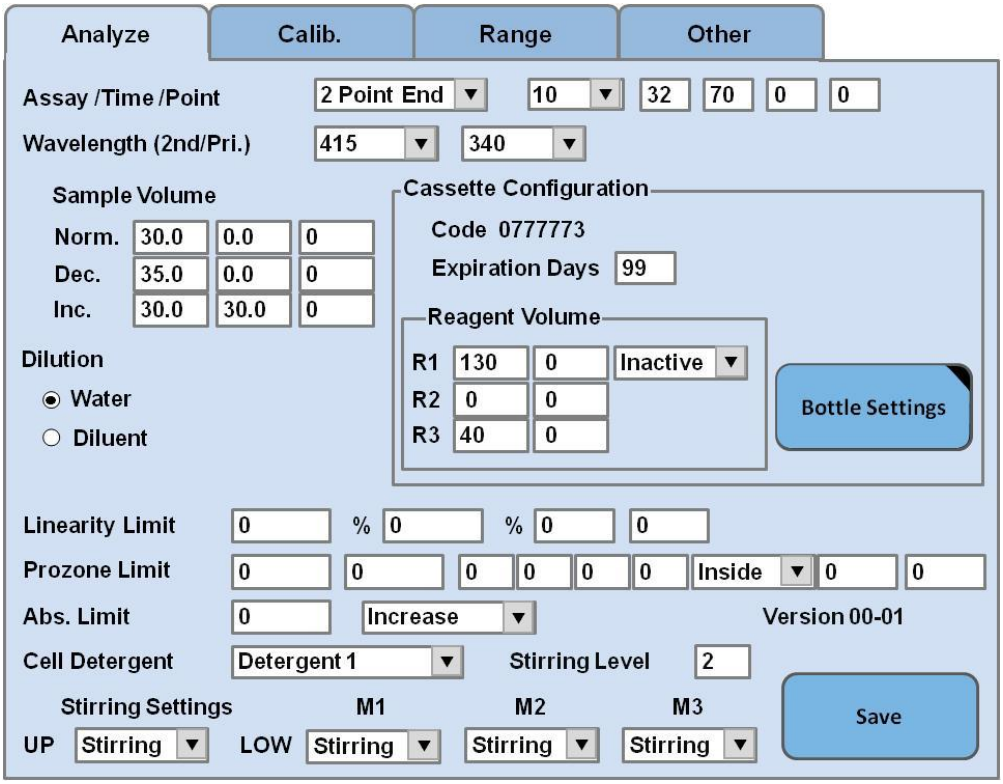
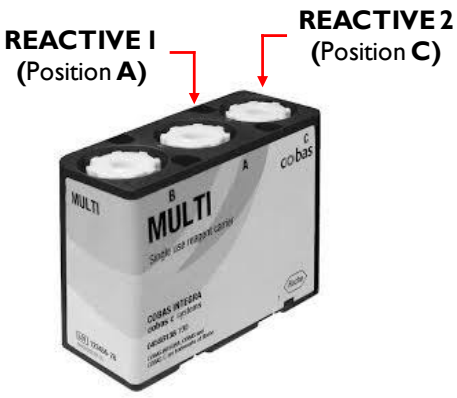
www.immunostep.com

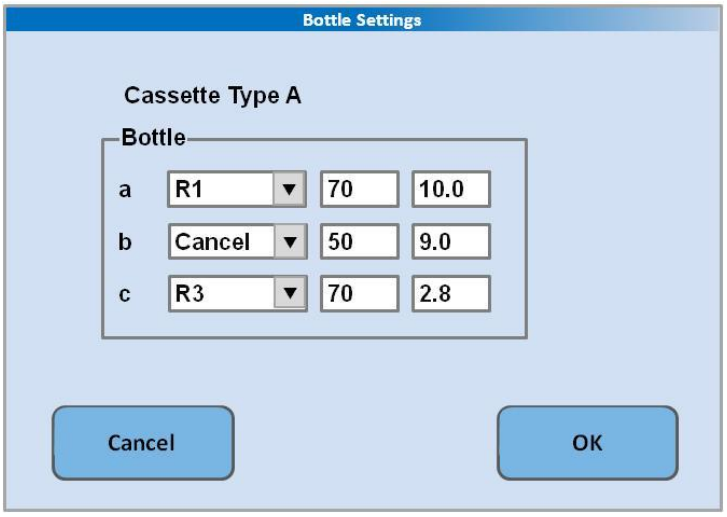
ANNEX – APPLICATION PARAMETERS

The following parameters were optimized in a Roche Cobas c502 automated analyzer:

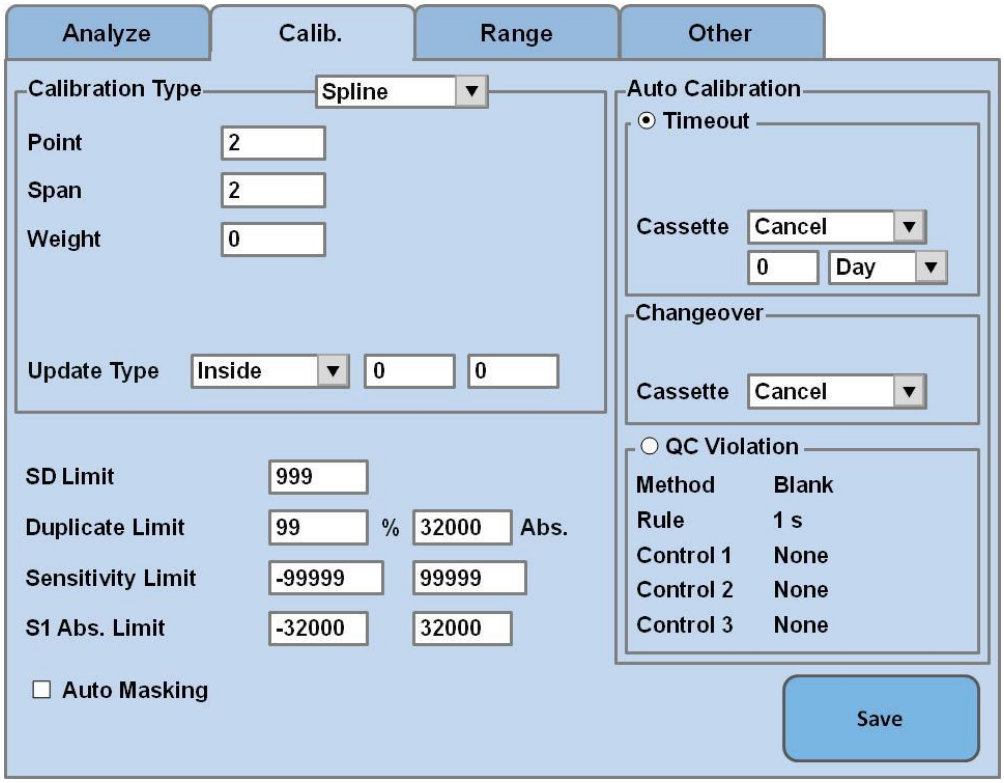


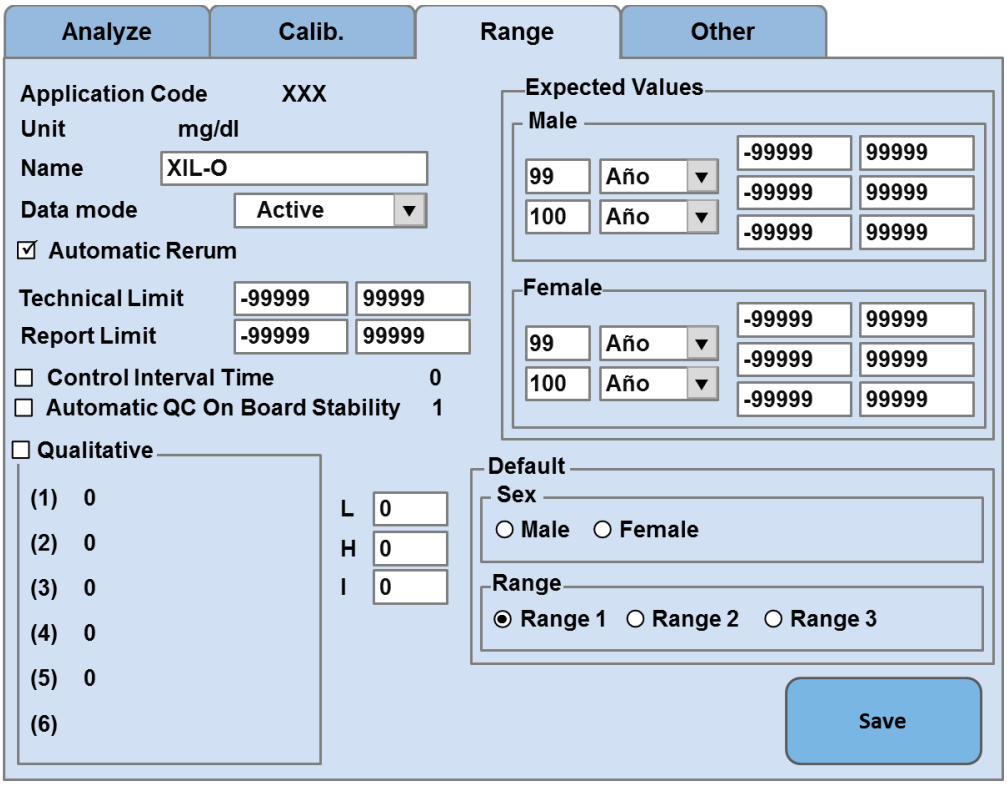
Cobas C Pack MULTI
(empty reagent cartridge)





Reagent cartridge for open channel tests in Cobas equipment from ROCHE (Cobas c Pack MULTI, Roche reference: 04593138190). In the case of XYLOSSAY®, **Reagent 1** must be in **position A**, and **Reagent 2** in **position C**.


Analyze
Calib.
Range
Other
Calibration Type
Spline
Point
2
Span
2
Weight
0
Update Type
Inside
0
0
SD Limit
999
Duplicate Limit
99
%
32000
Abs.
Sensitivity Limit
-99999
99999
S1 Abs. Limit
-32000
32000
Auto Masking
Auto Calibration
Timeout
Cassette
Cancel
0
Day
Changeover
Cassette
Cancel
QC Violation
Method
Blank
Rule
1 s
Control 1
None
Control 2
None
Control 3
None
Save


Analyze
Calib.
Range
Other
Application Code
XXX
Unit
mg/dl
Name
XIL-O
Data mode
Active
Automatic Rerum
Technical Limit
-99999
99999
Report Limit
-99999
99999
Control Interval Time
0
Automatic QC On Board Stability
1
Qualitative
(1) 0
(2) 0
(3) 0
(4) 0
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L
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Expected Values
Male
99
Año
100
Año
-99999
99999
-99999
99999
-99999
99999
Female
99
Año
100
Año
-99999
99999
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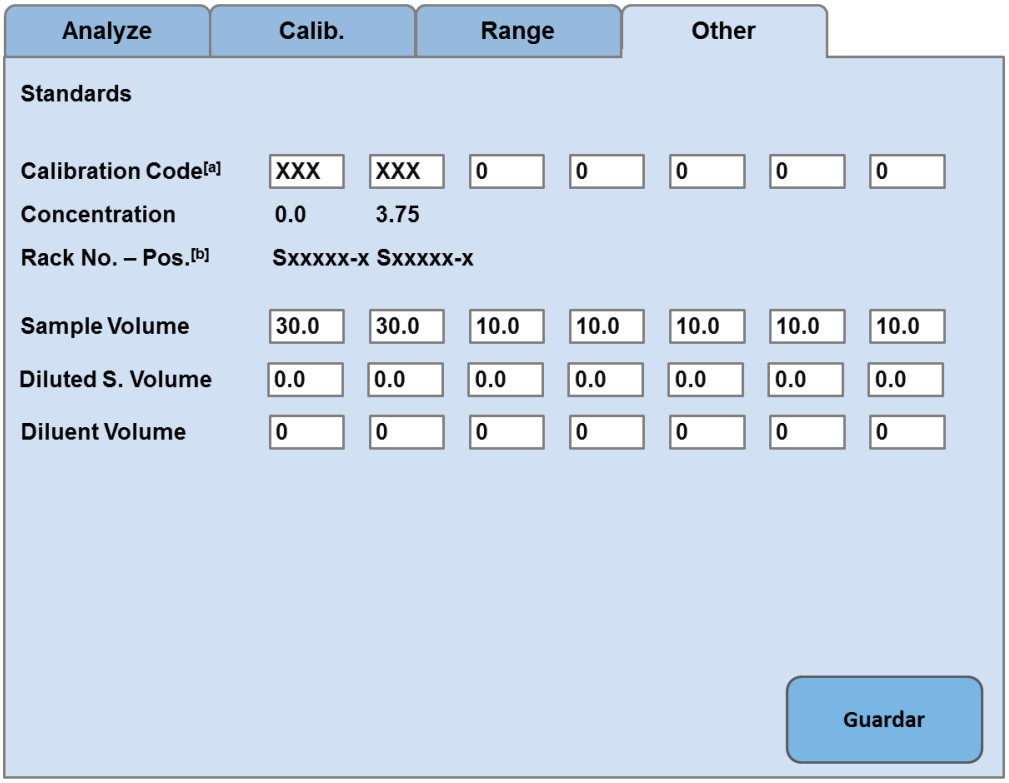
99999
Default
Sex
Male
Female
Range
Range 1
Range 2
Range 3
Save



[a] Configure in the Calibration Main Menu, Install Screen, and "Add Calibrator" window.
[b] Configure in the Calibration Main Menu, Calibrators Screen, and "Assign Calibrator" window.