Removal of Glucose excess in samples
Reagents for 32 samples

For in vitro use only
Store between +2 and +8°C

Principle

Several Sugar tests from the Roche Enzymatic product line are based on the measurement of Glucose, which means that free–Glucose must be measured separately and subtracted (Lactose / Glucose, Sucrose / Glucose, Maltose and Starch).

These tests do not work properly when the sample contains a large excess of Glucose, because the difference between the two cuvettes will be low and not reproducible (Δ A < 0.100). For this reason, the instructions for use of these assays include a special procedure to remove the Glucose excess in the sample, by using the enzymes Glucose Oxidase (GOD) and catalase:

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{D-Gluconate} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The present kit contains the reagents that are needed to apply this procedure on 32 samples.

Reagents

1: Reagent 1 (buffer): 1 bottle, approx. 70 ml

2: Reagent 2 (GOD): 1 bottle (red cap), approx. 3.5 ml

3: Catalase: 1 bottle (black cap), approx. 1.0 ml

4: Control sample: 1 bottle (white cap), approx. 10 ml (Glucose 25 g/L, Lactose 1 g/L, Sucrose 1 g/L)

All reagents are ready for use. These reagents are stable at 2-8 °C up to the expiry date shown on the package. Let the reagents reach the laboratory temperature (20 – 25°C) before use. Mix kindly before pipetting. Close immediately after handling.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Application examples

Determination of Lactose in Lactose-free samples (on the basis of German law § 64 - L 01.00-90)

This method is suited for liquid or solid samples as well, because Carrez clarification comes first, followed by Glucose oxidation:

- For solid samples, take a representative amount (50 – 100 g), grind and homogenize it carefully (sieve when necessary).
- Weigh precisely around 25 g milk into a beaker with 25 ml water and mix. For cheese or yogurt, weigh precisely 15 – 20 g sample as prepared above and add to 30 ml water, warm-up to 50°C and mix during 20 min.
- Add and mix after each step the Carrez solutions: 5 ml Carrez-I (3.6 g $K_4[Fe(CN)_6]x3H_2O$ / 100 ml) and 5 ml Carrez-II (7.2 g $ZnSO_4\,x7H_2O$ / 100 ml).
- Add NaOH (1 M, approx. 3.5 ml) to adjust the pH to 7.0 to 7.5.
- Transfer the sample preparation to a 100 ml volumetric flask, fill-up to the mark, mix and filter.
- Pipette 5 ml from the filtrate into a 50 ml tube (e.g. Falcon). For the control (vial 4), pipette 1 ml sample and 4 ml of water.
- Subsequently add 2 ml triethanolamine buffer (reagent 1), 100 µl GOD solution (reagent 2), 10 µl Catalase and 2.890 ml water (alternatively, it is possible to add 2.840 ml water and 50 µl of H_2O_2 30%). In order to avoid foam, it is possible to add one drop of octanol-1.
- Mix carefully and incubate 3 h with gentle mixing (rotator).
- Heat the tube in boiling water for 15 min, then let it cool down.
- Mix the sample and perform the Lactose-Glucose test **with 500 µl sample**, 40 min for the reaction with ß–Galactosidase, 20 min for the Glucose reaction, factor 2 for calculation of the samples and factor 10 for the control.

Determination of Lactose in Lactose-free milk or yogurt (simplified application for liquid samples only)

Milk samples are liquid and ready to use. For yogurt, the liquid is extracted by centrifugation as following:

- homogenize the yogurt sample and distribute approx. 50 g yogurt in a 50 ml tube several times (e.g. 4 tubes x 50 g)
- centrifuge at 3500 g for 10 min. (2 - 8°C) and collect the supernatants of all tubes to reach > 10 ml sample in total
- transfer 10 ml sample into a 50 ml tube, add 5 ml dist. water and adjust pH to 7.6 with NaOH (1M); fill with dist. water up to 20 ml (this results into a 1:2 dilution of the sample)
- in the procedure below, the sample volume is increased to 2 ml so the final dilution of the sample stays at 1:10.

Glucose removal takes place with 3 steps (First incubation with GOD, then Carrez clarification and finally pH adjustment):

Pipette in 50 mL tubes	**Control sample (ml)**	**Milk (ml)**	**Yogurt (ml)**
Buffer (reagent 1)	2.000	2.000	2.000
Sample	1.000	1.000	2.000
GOD (reagent 2)	0.100	0.100	0.100
Catalase	0.010	0.010	0.010
Bidist. Water	3.890	3.890	2.890
Mix gently; incubate for 3 h on a horizontal shaker (300 rpm). *Incubation can be performed overnight**			
Heat 15 min at 100 °C (inactivation of enzymes), then cool down to room temperature			
For Carrez-clarification, mix thoroughly after each step:			
Carrez-I-solution	0.500	0.500	0.500
Carrez-II-solution	0.500	0.500	0.500
NaOH (0.1 M)	1.000	1.000	1.000
Centrifuge 10 min at 3000 g and/or filter Transfer 4.5 ml supernatant in a 10 ml tube and add:			
HCl (0.1 M)	0.500	0.500	0.500
Mix and perform the Lactose/Glucose test **with 500 µl sample,** (reduce H_2O accordingly), 40 min incubation for ß–Galactosidase, 20 min for the Glucose reaction and factor 10 for calculation of results**.			

* For Sucrose/Glucose testing, the incubation is limited to 3 h: with overnight incubation, part of the Sucrose is lost because a side activity of the GOD causes a partial hydrolysis.

** For Sucrose/Glucose, the test is performed with 500 µl sample, 40 min incubation for ß-Fructosidase, 20 min for the Glucose reaction and factor 10 for calculation of results. An application for honey samples is available on request.

Notes

- The lowest detection limit of the Lactose-Glucose assay is 7 mg/l with v = 0.500 ml and ΔA = 0.020. Since the sample is diluted 1:10 during preparation, the limit is 70 mg/l (< 0.01%).
- The Lactose-Glucose test shows cross-reactions with Galacto-oligosaccharides (GOS), which might be present in Lactose-reduced milk. In this case it is not possible to quantify the remaining lactose below 0.03 %.