

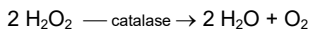
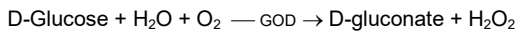
Removal of glucose excess in foodstuffs and other sample materials
Reagents for 32 determinations

For *in vitro* use only
Store between 2 - 8 °C

1. Test principle

Several sugar tests from the Enzytec™ *Liquid* product line are based on the measurement of glucose, which means that free glucose must be measured separately and subtracted (Enzytec™ *Liquid* Lactose/D-Glucose (E8130), Enzytec™ *Liquid* Sucrose/D-Glucose (E8180), and Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose (E8170)).

These tests do not work properly when the sample contains a large excess of glucose, because the ΔA difference between the two cuvettes will be low and not reproducible ($\Delta A < 0.100$). 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:



The present Enzytec™ Glucose Remover test kit contains the reagents that are needed to apply this procedure on 32 samples.

2. Reagents

2.1. Content & composition

The samples processed with the Enzytec™ Glucose Remover are suitable for manual and automated processing. The reagents are sufficient for 32 determinations.

- Reagent 1 (buffer): 1 bottle, approx. 70 mL
- Reagent 2 (GOD): 1 bottle (red cap), approx. 3.5 mL
- Catalase: 1 bottle (black cap), approx. 1.0 mL
- Control sample: 1 bottle (white cap), approx. 10 mL (glucose 25 g/L, lactose 1 g/L, sucrose 1 g/L)

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Application examples

3.1. Determination of lactose in lactose-free samples (on the basis of German law § 64-method L 01.00-90)

This method is suited for liquid, solid or viscous samples, because Carrez clarification comes first, followed by glucose oxidation:

- For solid samples, take a representative amount of sample (50 - 100 g), grind and homogenize it carefully.
- Weigh precisely around 25 g milk into a beaker with 25 mL dist. water and mix. For cheese or yogurt, weigh precisely 15 - 20 g sample as prepared above, add 30 mL water and mix, then warm-up to 50 °C for 20 min.
- Add and mix after each step the Carrez solutions: 5 ml Carrez-I (3.6 g $K_4[Fe(CN)_6] \times 3 H_2O$ / 100 mL) and 5 ml Carrez-II (7.2 g $ZnSO_4 \times 7 H_2O$ / 100 mL).
- Add 1 M NaOH (approx. 3.5 mL) to adjust the pH to 7.0 to 7.5.

- Transfer the sample to a 100 mL volumetric flask, fill-up to the mark with dist. water, mix and filter.
- Pipette 5 mL from the filtrate into a 50 mL tube. For the control sample (vial 4), pipette 1 mL sample and 4 mL dist. water.
- Subsequently add 2 mL triethanolamine buffer (reagent 1), 100 μ L GOD solution (reagent 2), 10 μ L catalase and 2.890 mL dist. water (alternatively, it is possible to add 2.840 mL water and 50 μ L H_2O_2 30 %). In order to avoid foam, it is possible to add one drop of octanol-1.
- Close the tube, mix carefully and incubate 3 h with gentle mixing (horizontal rotator).
- Heat the tube in boiling water for 15 min and then let it cool down.
- Mix the sample; perform the subsequent determination in the respective Enzytec™ *Liquid* test kit.

3.2. 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 more than 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.

The glucose removal takes place with three steps (first incubation with GOD, then Carrez clarification and finally the 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 and incubate for 3 h on a horizontal shaker (300 rpm). <i>Incubation can be performed overnight*.</i>			
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 the sample. Perform the respective Enzytec™ <i>Liquid</i> test and use a factor of 10 (due to sample preparation) for the calculation.			

* For Sucrose/D-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.

4. Controls & acceptance criteria

The efficiency of glucose degradation in lactose-free milk is $\geq 95\%$.
The control sample included in the kit should be carried along for quality control during each run.
The recovery of the control sample should be within $100 \pm 5\%$ for D-glucose and within $100 \pm 10\%$ for lactose and sucrose.

5. Performance data

5.1. Specificity & side activities

The Lactose/D-Glucose test shows cross-reactions with galactooligosaccharides (GOS), which may be present in low-lactose milk. In this case it is not possible to quantify the lactose residue below 0.03 %.

5.2. Linearity, measuring range & sensitivity

Refer to the respective Enzytec™ *Liquid* test kit descriptions and validation reports.

6. Automation with Pictus 500

Samples processed with the Enzytec™ Glucose Remover can also be automated in the respective Enzytec™ *Liquid* test kit.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link

<https://eifu.r-biopharm.com/>



8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

This information corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.