ENZYTEC™ *fluid* Glycerol — Id-N°: 5360

Test kit for 4 x 10 determinations — For in vitro use only

Reagent for photometric determination of Glycerol in food stuff and other sample material

Method

Enzymatic UV-Test with Glycerokinase (GK), ADP dependent Hexokinase (ADP-HK) and Glucose-6-phosphate-Dehydrogenase (G6P-DH).

Principle

Glycerol + ATP --- GK ---> L-Glycerol-3-phosphate + ADP

ADP + D-Glucose --- ADP-HK ---> D-Glucose-6-phosphate + AMP

D-Glucose-6-phosphate + NAD^+ --- G6P-DH --->

Gluconate-δ-lacton-6-phosphate + NADH + H^+

Storage instructions and reagent stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8°C and if contamination is avoided. Do not freeze!

Warnings and precautions

1. The reagents contain sodium azide (0.95 g/l) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

Reagent preparation

The reagents and the standard are ready-to-use.

Materials required but not provided

Distilled water (aseptic, free from heavy metals) and general laboratory equipment.

Package content and concentration of the reagents

R1	**4 x 20.8 ml**	Buffer	pH 7.5
		D-Glucose	10 mmol/l
		GK	≥ 500 U/l
		ADP-HK	≥ 500 U/l
		G6P-DH	≥ 5000 U/l
R2	**4 x 5.5 ml**	Buffer	pH 6.0
		NAD	≥ 20 mmol/l
		ATP	≥ 1 mmol/l

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure.

- Use clear, colourless and practically neutral liquid samples directly, or after dilution to a Glycerol concentration between 40 – 260 mg/l.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples. Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water. Filtrate or clarify if necessary.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter.Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly coloured samples with Polyvinylpolypyrrolidone (PVPP e.g. 1 g/100 ml Sample), or measure instead with reagent blank (RB) each sample with sample blank (SB). For preparation see assay procedure.

Carrez clarification:

Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium–hexacyanoferrat-(II)-trihydrat, 85 mmol = 3.60 g $K_4[Fe(CN)_6]$ × 3 H_2O/100 ml), 5 ml Carrez-II-solution (zinc sulphate, 250 mmol = 7.20 g $ZnSO_4$ × 7 H_2O/100 ml) and 10 ml 0.1 M NaOH. Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

Assay procedure

Application sheets for automated systems are available on request.

Wavelength: 340 nm, Hg 334 nm, Hg 365 nm
Optical path: 1 cm
Temperature: 20 – 25 °C / 37 °C
Measurement: against or against water

For the manual procedure below, reagent blank must be performed for every run, and subtracted during calculation of results. Sample blank is performed only when interferences by the sample itself are suspected.

	Reagent Blank (RB)	Sample	Sample Blank (SB, optional)
Sample / Standard	-	100 µl	100 µl
Dist. water	100 µl	-	-
Reagent 1	2000 µl	2000 µl	2000 µl
Mix, incubate for 1 min. at 37 °C or 3 min. at 20 - 25 °C, read absorbance A1, then add:			
Reagent 2	500 µl	500 µl	-
Dist. water	-	-	500 µl
Mix, wait till the end of the reaction (incubation for approx. 5 min. at 37 °C or approx. 15 min. at 20 - 25 °C) and read absorbance A2.			

Calculation

Measurement with RB: $\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$

or with SB: $\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{SB} - (A_2 - df \times A_1)_{RB}$

With df = dilution factor of optical densities, because of reagent volumes:

df = (sample volume + R1) / (sample volume + R1 + R2) = 0.808.

Calculation formula:

$$C_{Glycerol}\ [g/l\ sample\ sol.] = \frac{V \times MW \times \Delta A}{\varepsilon \times d \times v \times 1000}$$

with:

V	(Total volume)	= 2600	[µl]
MW	(Molecular weight)	= 92.1	[g/mol]
d	(Optical path)	= 1.00	[cm]
v	(Sample volume)	= 100	[µl]
ε	(Extinction coefficient NADH)	[l x $mmol^{-1}$ x cm^{-1}] :	
	340 nm = 6.3	334 nm = 6.18	365 nm = 3.4

Here from results for the determination at:

340 nm:	$c_{Glycerol}$ [g/l]	= 0.380 x ΔA
334 nm		= 0.388 x ΔA
365 nm		= 0.704 x ΔA

The above factors have to be recalculated again when changing parameters, e.g. the sample volume.
Dilution factors of the sample preparation have to be considered in the calculation.

Calculation in solid samples:

$$Content_{Glycerol}\ [g/100\ g] = \frac{C_{Glycerol}\ [g/l\ ]}{Weight_{sample}\ [g/l\ sample\ solution]} \times 100$$

Calibration / assay control

For the calibration of automated photometric systems and for internal quality control of precision and accuracy use the Enzytec Fluid Glycerol standard (Cat. N°. 5480, 3 x 3 ml). The standard is ready-to-use.

Performance characteristics

Measuring range

The test has been developed to determine Glycerol concentrations within a measuring range from 10 – 250 mg/l (measured at 340 nm). When values exceed this range samples should be diluted into the range with dist. water. The dilution factor has to be considered in the calculation.

Specificity

The determination is specific for Glycerol. Due to the low GK activity Dihydroxyacetone is not converted.

Lowest detection limit

1.0 mg/l, measured at 340 nm.
The lowest detection limit corresponds to the smallest Glycerol concentration differentiating from zero. It is calculated out of three standard deviations from 20 replicates of a zero sample.

Waste management

Please refer to local legal requirements.

Manufacturer

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