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ProductInformation

Free Glycerol Reagent

Catalog Number **F6428**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Free Glycerol Reagent is for the quantitative enzymatic determination of glycerol in serum or plasma at 540 nm.

Triglycerides, esters of fatty acids and glycerol,[1] do not circulate freely in plasma, but are bound to proteins and transported as macromolecular complexes called lipoproteins.[2] Methods for triglycerides determination generally involve enzymatic[3] or alkaline[4] hydrolysis of triglycerides to glycerol and free fatty acids followed by either chemical or enzymatic measurement of the glycerol released. The Serum Triglyceride Determination Kit (Catalog Number TR0100) can be used for the measurement of glycerol, true triglycerides, or total triglycerides in serum or plasma. The procedure involves enzymatic hydrolysis by lipase of the triglycerides to glycerol and free fatty acids.[5] The glycerol produced is then measured by coupled enzyme reactions. Many of the triglyceride reagents which are commercially available, do not differentiate between endogenous glycerol and glycerol derived by hydrolytic action of lipase on glycerides.

The Free Glycerol Reagent measures free, endogenous glycerol using the same coupled enzyme reactions without the initial lipase hydrolysis. Glycerol is phosphorylated by adenosine-5′-triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5′-diphosphate (ADP) in the reaction catalyzed by glycerol kinase (GK). G-1-P is then oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Peroxidase (POD) catalyzes the coupling of H_2O_2 with 4-aminoantipyrine (4-AAP) and sodium *N*-ethyl-*N*-(3-sulfopropyl) *m*-anisidine (ESPA) to produce a quinoneimine dye that shows an absorbance maximum at 540 nm.[6,7] The increase in absorbance at 540 nm is directly proportional to the free glycerol concentration of the sample.

Glycerol Assay Enzymatic Reactions:

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{G-1-P} + \text{ADP}$$

$$\text{G-1-P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{ESPA} \xrightarrow{\text{POD}} \text{Quinoneimine Dye} + H_2O$$

Components

1 vial is sufficient for 50 reactions

Free Glycerol Reagent (Catalog Number F6428)	40 ml
After reconstitution, the solution will have the following approximate concentrations:	
ATP	0.75 mM
Magnesium salt	3.75 mM
4-Aminoantipyrine	0.188 mM
N-Ethyl-*N*-(3-sulfopropyl) *m*-anisidine, sodium salt	2.11 mM
Glycerol kinase (microbial)	1,250 units/L
Glycerol phosphate oxidase (microbial)	2,500 units/L
Peroxidase (horseradish)	2,500 units/L
Buffer	pH 7.0 ± 0.1
Sodium azide, added as preservative	0.05%
Nonreactive stabilizers and fillers	

Reagents and Equipment Required but Not Provided

- Spectrophotometer capable of accurately measuring absorbance at 540 nm
- Glycerol Standard Solution (Catalog Number G7793). The Glycerol Standard Solution concentration is 0.26 mg glycerol/ml, with an equivalent triolein concentration of 2.5 mg/ml.
- Test tubes or cuvets
- Pipetting devices for the accurate delivery of volumes required for the assays
- Timer
- A constant temperature water bath, if the assay is to be performed at a temperature other than ambient

Storage and Stability

Store the unreconstituted Free Glycerol Reagent at 2–8 °C. The reagent is stable for 24 months after manufacture. See product label for actual expiration date.

The reconstituted Free Glycerol Reagent is stable for 60 days when stored at 2–8 °C or for 5 days at room temperature (18–26 °C).

The Free Glycerol Reagent is not suitable for use if the absorbance of freshly prepared solution exceeds 0.4, when measured in 1 cm lightpath at 540 nm versus water as reference. Discard the vial if the dry reagent exhibits caking due to possible moisture penetration, does not dissolve completely upon reconstitution, or if the solution appears turbid.

Precautions and Disclaimer

The Free Glycerol Reagent is for R&D use only, not for *in vitro* diagnostic use, drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Use ultrapure deionized water for the preparation of the reagent. Water, cell culture tested (Catalog Number W3500), is recommended to ensure good assay performance.

Free Glycerol Reagent

Reconstitute the Free Glycerol Reagent with 40 ml of water. After addition of water, stopper the vials, and immediately mix several times by inversion. DO NOT SHAKE. Protect the reagent from light by storing in amber bottles.

Sample Collection and Preparation

It is recommended that sample collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious. Samples collected either in plain tubes or in those containing anticoagulant, preferably EDTA or heparin, are centrifuged to obtain serum or plasma. Samples should not be left exposed at room temperature for more than 48 hours to prevent bacterial contamination.

Interfering Substances

Avoid use of extremely hemolyzed and icteric samples. Glycerol contamination in tubes, stoppers, and glassware will interfere in the assay.[9] If high A_{540} readings are obtained with the Free Glycerol Reagent, check the quality of the water used to reconstitute the reagents.

Procedure

1. Prepare the Free Glycerol Reagent according to the preparations instructions.
2. Set the spectrophotometer wavelength to 540 nm and the absorbance reading to zero with water as the reference.
3. Warm the reconstituted Free Glycerol Reagent to assay temperature.
4. Set up a series of labeled cuvets for Blank, Standard, and Sample.
5. Pipette 0.8 ml of the Free Glycerol Reagent into each cuvet.
6. Add 10 µl (0.01 ml) of water, Glycerol Standard (Catalog Number G7793), and sample to cuvets labeled Blank, Standard, and Sample, respectively. Mix by gentle inversion.
7. Incubate for 5 minutes at 37 °C.
   Note: Incubate ambient temperature assays for 15 minutes and assays at 30 °C for 10 minutes.
8. Read and record absorbance (A_{540}) of Blank, Standard, and Sample versus water as reference.
9. Calculate the glycerol concentration of the sample.

Calculation:

Glycerol content =

$$\frac{(A_{SAMPLE} - A_{BLANK})}{(A_{STANDARD} - A_{BLANK})} \times \text{Concentration of Standard}$$

Glycerol content may be calculated in terms of glycerol or in terms of equivalent triolein concentration.

References

1. Tietz, N.W., in Fundamentals of Clinical Chemistry, Saunders (Philadelphia, PA: 1970), p. 329.
2. Kaplan, L.A., and Pesce, A.J., in Clinical Chemistry Theory, Analysis, and Correlation, CV Mosby Co. (St. Louis, MO: 1984), p. 567.
3. Buccolo, G., and David, H., Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem., **19**, 476 (1973).
4. Eggstein, M., and Kreutz, F.H., Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe. Klin. Wschr., **44,** 262 (1966).
5. McGowan, M.W., *et al*., A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., **29**, 538 (1983).
6. Trinder, P., Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., **6**, 24 (1969).
7. Barham, D., and Trinder, P., An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, **97**, 142 (1972).
8. Tietz, N.W., *et al*., An improved method for the determination of lipase in serum. Am. J. Clin. Pathol., **31,** 148 (1959).
9. Chowdhury, F.R., *et al*., Glycerol-like contamination of commercial blood sampling tubes. J. Lipid Res., **12,** 116 (1971).

Related Products

- Serum Triglyceride Determination Kit, Catalog Number TR0100
  For the determination of total triglycerides and free glycerol
- Free Glycerol Determination Kit, Catalog Number FG0100
  For the determination of free glycerol
- Triglyceride Reagent, Catalog Number T2449
  To be used to supplement the Free Glycerol Reagent for the determination of total triglycerides
- Glycerol Standard Solution, Catalog Number G7793
- Water, sterile-filtered, cell culture tested, Catalog Number W3500

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