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ProductInformation

Serum Triglyceride Determination Kit

Catalog Number **TR0100**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Serum Triglyceride Determination Kit is for the quantitative enzymatic measurement of glycerol, true triglycerides, and total triglycerides 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 triglyceride 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.

This 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 other commercially available triglyceride reagents do not differentiate between endogenous glycerol and glycerol derived by hydrolytic action of lipase on glycerides.

Triglyceride Assay Enzymatic Reactions:

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol + Fatty acids}$$

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

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

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

Triglycerides are first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol is then 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 triglyceride concentration of the sample.

Free, endogenous glycerol may be measured using the same coupled enzyme reactions without the initial lipase hydrolysis. The reaction sequence would be the same except for the lipolytic reaction. The increase in absorbance at 540 nm is then directly proportional to glycerol concentration of the sample.

Components

One kit is sufficient for 250 total triglyceride tests. The kit also includes sufficient reagent for an additional 250 free glyceride tests for true triglyceride determination using Procedure B. After reconstitution, the prepared solutions will have the following approximate concentrations:

- Triglyceride Reagent 5 × 10 ml
  (Catalog Number T2449)
  - 250,000 units/L Lipase (microbial)
  - 0.05% Sodium azide, added as preservative
  - Nonreactive stabilizers and fillers
- Free Glycerol Reagent 10 × 40 ml
  (Catalog Number F6428)
  - 0.75 mM ATP
  - 3.75 mM Magnesium salt
  - 0.188 mM 4-Aminoantipyrine
  - 2.11 mM *N*-Ethyl-*N*-(3-sulfopropyl) *m*-anisidine, sodium salt
  - 1,250 units/L Glycerol Kinase (microbial)
  - 2,500 units/L Glycerol Phosphate Oxidase (microbial)
  - 2,500 units/L Peroxidase (horseradish)
  - Buffer, pH 7.0 ± 0.1
  - 0.05% Sodium azide, added as preservative
  - Nonreactive stabilizers and fillers

Reagents and Equipment Required but Not Provided

- Spectrophotometer capable of accurately measuring absorbance at 540 nm
- Test tubes or cuvets
- Pipettes 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 non-ambient temperature
- Glycerol Standard Solution, 2.5 mg/ml equivalent triolein concentration (Catalog Number G7793)

Storage and Stability

Store the Free Glycerol Reagent and Triglyceride Reagent at 2–8 °C prior to reconstitution. The reagents are stable for 24 months after manufacture. See product labels for actual expiration date.

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

The Triglyceride Working Reagent will remain stable for 60 days at 2–8 °C and 5 days at room temperature (18–26 °C).

The triglyceride reagents are not suitable for use if the absorbance of freshly prepared Free Glycerol Reagent exceeds 0.4 when measured in 1 cm light path at 540 nm versus water as the reference. Discard the vials if the dry reagents exhibit caking due to possible moisture penetration, do not dissolve completely upon reconstitution, or if the solutions appear turbid.

Precautions and Disclaimer

The Serum Triglyceride Determination Kit 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 reagents. Water, cell culture tested (Catalog Number W3500), is recommended to ensure good assay performance.

Free Glycerol Reagent and Triglyceride Reagent:
Reconstitute the Free Glycerol Reagent and the Triglyceride Reagent with 40 ml and 10 ml of water, respectively. After addition of water, stopper the vials, and immediately mix several times by inversion. DO NOT SHAKE. Protect the reagents from light by storing in amber bottles.

Triglyceride Working Reagent:
Prepare the Triglyceride Working Reagent by combining 4 ml of the reconstituted Free Glycerol Reagent with 1 ml of the reconstituted Triglyceride Reagent, or by mixing similar proportions of both reagents. Store the Triglyceride Working Reagent in an amber bottle.

Triglyceride Reagent Blank:
Prepare the Triglyceride Reagent Blank by combining 4 ml of the reconstituted Free Glycerol Reagent with 1 ml of water or similar proportions of the reconstituted Free Glycerol Reagent and water. Store the reagent in an amber container.

Standard:
The Glycerol Standard (Catalog Number G7793) is a ready-to-use solution and may be used to generate a standard curve. The Triglyceride Reagent is linear up to 10 mg/ml.

Sample Collection and Preparation:
Samples should be collected 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. Triglycerides in such samples are reportedly stable for up to one week at 2–8 °C or 48 hours at room temperature.[8] Samples should not be left at room temperature for more than 48 hours in order to avoid bacterial contamination.

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

Procedures

A. Determination of Glycerol, True Triglycerides, and Total Triglycerides

1. Prepare the Free Glycerol Reagent and the Triglyceride Reagent according to the preparation instructions.
2. Set the spectrophotometer wavelength to 540 nm and the absorbance reading to zero with water as the reference.
3. Warm the Free Glycerol Reagent and the Triglyceride 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, 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 initial absorbance (IA) of Blank, Standard, and Sample at 540 nm versus water as the reference.
9. Add 0.2 ml of the reconstituted Triglyceride Reagent to each cuvet, mix, and continue incubation at 37 °C for 5 more minutes (or for 15 and 10 more minutes if assay is performed at ambient temperature or 30 °C, respectively).
10. Read and record final absorbance (FA) of Blank, Standard, and Sample at 540 nm versus water as the reference.
11. Calculate the concentrations of glycerol, true triglycerides, and total triglycerides in the sample.

Calculations:

Total Triglyceride Concentration in Serum or Plasma:

Total Serum Triglyceride Concentration (equivalent triolein concentration) =

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

Glycerol in Serum or Plasma:

Glycerol Concentration (equivalent triolein concentration) =

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

True Serum Triglyceride Concentration:

True Serum Triglyceride Concentration (equivalent triolein concentration) =

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

Where F = 0.81/1.01 = 0.80

Alternative Procedure B

Determine total serum triglyceride concentration and serum glycerol concentration as described below. Subtract the serum glycerol concentration from the total serum triglyceride concentration to obtain true serum triglyceride concentration.

B1. Determination of Total Triglyceride Concentration:

1. Prepare the Triglyceride Working Reagent according to the preparation instructions.
2. Set the spectrophotometer wavelength to 540 nm and the absorbance reading to zero with water as the reference.
3. Warm the Triglyceride Working Reagent to assay temperature.
4. Set up a series of labeled cuvets for Blank, Standard, and Sample.
5. Pipette 1.0 ml of the Triglyceride Working Reagent into each cuvet.
   Note: If the spectrophotometer accommodates larger volumes, use 3 ml of the Triglyceride Working Reagent and 30 µl of sample.
6. Add 10 µl (0.01 ml) of water, Glycerol Standard, 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) of Blank, Standard, and Sample at 540 nm versus water as the reference.
9. Subtract absorbance of the Blank from absorbance of the Standard and the Sample to obtain change in absorbance (A) due to triglycerides.
10. Calculate the total triglyceride concentration of sample.

Calculation:

Total Triglyceride Concentration in Serum or Plasma:

Total Serum Triglyceride Concentration (equivalent triolein concentration) =

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

B2. Determination of Glycerol Concentration:

1. Prepare the Triglyceride Reagent Blank according to the preparation instructions.
2. Set the spectrophotometer wavelength to 540 nm and the absorbance reading to zero with water as the reference.
3. Warm the Triglyceride Reagent Blank to assay temperature.
4. Set up a series of labeled cuvets for Blank, Standard, and Sample.
5. Pipette 1.0 ml of the Triglyceride Reagent Blank into each cuvet.
   Note: If the spectrophotometer accommodates larger volumes, use 3 ml of the Triglyceride Reagent Blank and 30 µl of sample.
6. Add 10 µl (0.01 ml) of water, Glycerol Standard, 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) of Blank, Standard, and Sample at 540 nm versus water as the reference.
9. Subtract absorbance of the Blank from absorbance of the Standard and the Sample to obtain change in absorbance (A) due to glycerol.
10. Calculate the glycerol concentration of sample.

Calculations:
Glycerol Content in serum or plasma:

Glycerol Content (equivalent triolein concentration) =

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

True Serum Triglyceride Concentration:
Subtract serum glycerol content from total serum triglyceride concentration to determine true serum triglyceride concentration (mg/ml, expressed as equivalent triolein concentration).

True Serum Triglyceride Concentration (mg/ml) =
Total Serum Triglyceride Concentration (mg/ml) – Serum Glycerol Content (mg/ml)

Millimolar Concentration:
To convert the equivalent triolein concentration from mg/ml into mM, multiply the concentration (mg/ml) by 1.13.

Limitations
The Triglyceride Reagent is linear up to 10 mg/ml. If the sample triglyceride concentration exceeds this value, dilute 1 part sample with 1 part isotonic saline and reassay. Multiply result by 2 to compensate for dilution.

References

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6. Trinder, P., Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., **6**, 24 (1969).
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9. Chowdhury, F.R., *et al*., Glycerol-like contamination of commercial blood sampling tubes. J. Lipid Res., **12,** 116 (1971).

Related Products

Free Glycerol Determination Kit,
Catalog Number FG0100
Free Glycerol Reagent, Catalog Number F6428
Triglyceride Reagent, Catalog Number T2449
Glycerol Standard Solution, Catalog Number G7793
Water, sterile-filtered, cell culture tested,
Catalog Number W3500

VNC,MAM,DG,KTA 11/06-1