



TRIGLYCERIDES REAGENT
C115-0A, C115-0B, V115-12

Contents	Product No.	Package
Triglycerides GPO Reagent Triglycerides Reagent Triglycerides Diluent	C115-0A C115-01 C115-02	6 x 0.375g 6 x 25 mL
Triglycerides GPO Reagent Triglycerides Reagent Triglycerides Diluent	C115-0B C115-03 C115-04	6 x 0.745g 6 x 50 mL
Triglycerides GPO Reagent Triglycerides Reagent Triglycerides Diluent	V115-12 V115-13 V115-14	6 x 0.194g 6 x 13 mL

REAGENT PREPARATION

Add contents of one bottle of diluent into one bottle of powder.
Mix gently until fully dissolved.

REAGENT STORAGE AND STABILITY

Unopened reagents are stable until the date stated on the label when stored at 2-8C.
After reconstitution the working reagent is stable in liquid form for at least
14 days when stored at 2-8C.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330



TRIGLYCERIDES GPO TRINDER C115-0A, C115-0B, V115-12 MANUAL/AUTOMATED PROCEDURE

Intended Use

For **IN VITRO quantitative** determination of Triglycerides in serum.

Clinical Significance

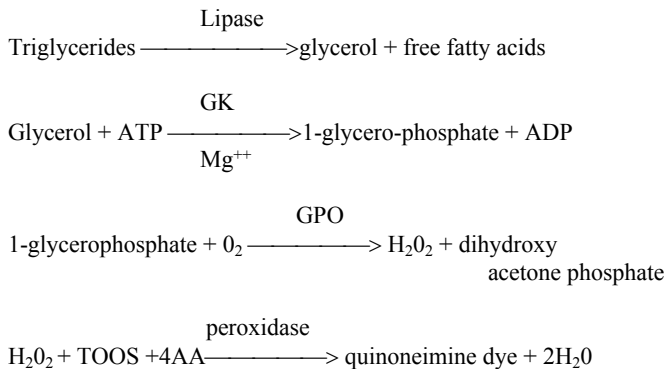
The determination of Triglycerides is primarily used for diagnosing atherosclerosis and heart disease, diabetes mellitus, nephritis, biliary obstruction and metabolic disorders associated with endocrine disturbances, as well as for monitoring the causes and treatments.

Method History

Various methods for the determination of Triglycerides in human serum are currently in use in the routine clinical laboratory. With the advent of stable, more specific lipolytic enzymes, simpler and more efficient procedures have been described. (1,2) The Catachem Triglycerides method described below uses glycerophosphate oxidase coupled with a sensitive colorimetric endpoint reaction based on the work of Trinder. (4)

Method Principle

Serum Triglycerides are hydrolyzed by microbial lipase to glycerol and free fatty acids. The resultant glycerol, in the presence of glycerol kinase (GK) ATP and Mg⁺⁺ ions is phosphorylated to glycerol-1-phosphate. This latter metabolite is then oxidized by glycerophosphate oxidase (GPO) to produce hydrogen peroxide. The hydrogen peroxide thus produced is quantitatively determined by coupling 4-aminoantipyrine with TOOS (N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methyl-aniline, sodium salt, dihydrate) where a quinonimine dye with maximum absorption at 545 nm is produced. The following reaction scheme illustrates the reactions that occur in this method:



Reagent Content

The concentration of the active ingredients in the reagent will be approximately as follows:

Triglycerides GPO-Trinder Reagent

One liter after reconstitution contains:

Buffer	
ATP	0.30 mM
4-aminoantipyrine	0.15 mM
TOOS	0.54 mM

Lipase	1000 U
Glycerol Kinase	1000 U
Glycerophosphate oxidase	3000 U
Peroxidase	2000 U

Precautions

Avoid contact of the reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH**

Preparation Of Working Reagent

Catachem Triglycerides Reagent is packaged as a power reagent with a separate diluent. To prepare the reagent mix one bottle of powder with one bottle of diluent.

Reagent Storage And Stability

Store the reagents at 2-8°C. When stored as directed, these reagents are stable until the expiration date stated on the label. Upon reconstitution the reagent is stable for at least 14 days when stored at 2-8°C. The Catachem Triglycerides reagent has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures of up to 40°C for up to one week.

Specimen Collection And Preparation

The use of clear, unhemolyzed serum is recommended. A fasting specimen is necessary for an accurate triglycerides determination.

Interfering Substances

A number of substances have been reported to affect the accuracy of triglycerides methods using oxidase-peroxidase procedures (3,2). A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al (6).

Expected Values

The "risk values" for Triglycerides in a human population as defined by the National Heart and Lung Institute are listed below. Levels in animals differ species to species.

AGE (Years)	TRIGLYCERIDES (mg/dL)
20-29	140
30-49	160
50-59	190

The normal range of this assay, as performed below, using human samples serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Procedure

Important: Read the entire instructions procedure before proceeding with the assay.



TRIGLYCERIDES GPO TRINDER
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MANUAL/AUTOMATED PROCEDURE

Materials Required But Not Provided

- Spectrophotometer
Cuvettes
Timer
Pipette
Pipette

Materials Provided

Catachem Triglycerides Reagent

Analytical Parameters

- Wavelength
Temperature
Pathlength
Reaction Mode
Reaction Time
Reagent Volume
Sample Volume
Total Volume
Sample-to-Reagent Ratio

Assay Procedure

- 1. After reconstitution pipette 1 ml of Catachem Triglycerides Reagent into 3 separate cuvettes labeled "Calibrator", "Sample", and "Blank"
2. Pipette 0.01 ml (10 uL) of Calibrator or Sample into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at 37°C.
4. Set spectrophotometer wavelength at 545 nm and zero the instrument with the cuvette marked "Blank".
5. Read the "Calibrator" and "Sample" absorbencies.
6. Calculate the Triglycerides concentration (mg/dL) in the Sample(s), as shown in "results and calculations" below.

Results And Calculations

Triglycerides (mg/dL) = (Sample Abs / Calibrator Abs) x Calibrator (mg/dL)

Example:

- Sample absorbance = 0.300
Calibrator absorbance = 0.250
Calibrator (mg/dL) = 200

Triglycerides (mg/dL) = (0.300 / 0.250) x 200 = 240 mg/dL

Quality Control

To ensure optimal performance of this reagent and this procedure, we recommend systematic calibration using Catachem's Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Method Performance Characteristics

Sensitivity: 0.0009-0013 absorbance units per mg/dL.

Linear Range: 0-1000 mg/dL.

Precision: Within-run and day-to-day precision is summarized below:

Triglycerides Precision Study

Table with 5 columns: TGS, Mean, SD, CV, Total Precision. Rows show values for 65, 103, 390, and 722 mg/dL.

Correlation

A comparison of the method using an automated analyzer and a reference method resulted in the following regression statistics:

- Range = 30-722 mg/dL
N = 118
Y = 1.03-5.6
r = 0.997
Sy.x = 7.0

References

- 1. Bucolo G, David, H. Clin Chem 20, 47648(1973).
2. Fossati P, Prencipe L. Clin Chem 28, 10 (1980).
3. McGowan MW, Artis JD, Strandbergh DR and Zak B. Clin Chem 29, 3 (1983).
4. Trinder P, Ann Clin Biochem 6, 24 (1969).
5. Katsumi Tamaoku, Keiuy Ueno, Kayoko Akiura and Yosuke Ohkura. Chem PharmBull 30 (7) 2492-2497 (1982).
6. Young DS, Pestaner LD, Gibberman V. Clin Chem 21, 5 (1975).