CATACHEM

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

Contents	Product No.	Package
Triglycerides GPO Reagent	**C115-0A**	
Triglycerides Reagent	C115-01	6 x 0.375g
Triglycerides Diluent	C115-02	6 x 25 mL
Triglycerides GPO Reagent	**C115-0B**	
Triglycerides Reagent	C115-03	6 x 0.745g
Triglycerides Diluent	C115-04	6 x 50 mL
Triglycerides GPO Reagent	**V115-12**	
Triglycerides Reagent	V115-13	6 x 0.194g
Triglycerides Diluent	V115-14	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
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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 lipolitic 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-mehylaniline,sodium salt, dihydrate) where a quinonemine dye with maximum absorption at 545 nm is produced. The following reaction scheme illustrates the reactions that occur in this method:

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[Mg^{++}]{\text{GK}} \text{1-glycero-phosphate + ADP}$$

$$\text{1-glycerophosphate + } 0_2 \xrightarrow{\text{GPO}} H_20_2 + \text{dihydroxy acetone phosphate}$$

$$H_20_2 + \text{TOOS +4AA} \xrightarrow{\text{peroxidase}} \text{quinoneimine dye + } 2H_20$$

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.

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MANUAL/AUTOMATED PROCEDURE

Materials Required But Not Provided

Spectrophotometer	
Cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.010 ml for sample

Materials Provided

Catachem Triglycerides Reagent

Analytical Parameters

Wavelength	545 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5 min
Reagent Volume	1.0 ml
Sample Volume	0.01 ml (10µL)
Total Volume	1.01 ml
Sample-to-Reagent Ratio	1:100

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

$$\text{Triglycerides (mg/dL)} = \frac{\text{Sample Abs}}{\text{Calibrator Abs}} \times \text{Calibrator (mg/dL)}$$

Example:

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

$$\text{Triglycerides (mg/dL)} = \frac{0.300}{0.250} \times 200 = 240 \text{ mg/dL}$$

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

TGS	Within-Run		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
65	1.0	1.5	1.6	2.2
103	0.5	0.5	1.7	1.6
390	1.2	0.3	3.0	0.8
722	3.8	0.6	7.9	1.1

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).

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