INTRODUCTION

1. AutoZyme NEW Triglycerides is a reagent set for determination of triglycerides **based on enzymatic method** using Lipoprotein lipase, Glycerol kinase, Glycerol phosphate oxidase and Peroxidase.
2. AutoZyme NEW Triglycerides is a **single reagent system.**
3. AutoZyme NEW Triglycerides is **a High Stability Reagent.**
4. Triglycerides can be determined in just **10 minutes.**
5. AutoZyme NEW Triglycerides is **linear** upto 800 mg% triglycerides.
6. AutoZyme NEW Triglycerides can be used on any **Colorimeter, Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
7. The influence of lipids, haemolysis and bilirubin (upto 8 mg%) is negligible.
8. The **shelf-life** of AutoZyme NEW Triglycerides is 17 months.

PRINCIPLE

Glycerol released from hydrolysis of triglycerides by lipoprotein lipase is converted by glycerol kinase into glycerol - 3 - phosphate which is oxidised by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red coloured compound.

Triglycerides $\xrightarrow{\text{LPL*}}$ **Fatty acids + Glycerol**

Glycerol + ATP $\xrightarrow{\text{GK*}}$ **Glycerol-3-phosphate + ADP**

Glycerol-3-phosphate $+O_2$ $\xrightarrow{\text{GPO*}}$ **Dihydroxyacetone phosphate** $+H_2O_2$

H_2O_2 **+ Phenolic chromogens** $\xrightarrow{\text{POD*}}$ **Red colour compound**

*Abbreviations

LPL = Lipoprotein Lipase
GK = Glycerol Kinase
GPO = Glycerol Phosphate Oxidase
POD = Peroxidase

PREPARATION OF WORKING SOLUTION

Reconstitute reagents as per instruction on individual bottle label to prepare working reagent.
Mix by gentle swirling or inversion. **Do not shake vigorously.**

REAGENT STORAGE & STABILITY & HANDLING

The reagent should be stored at 2 - 8°C and is stable till the expiry date indicated on the label.

Stability of reconstituted working reagent is printed on the bottle label.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Buffer, pH 6.8	50 mmol/l
• Lipase	≥ 2000 IU/l
• Glycerol kinase	≥ 300 IU/l
• Glycerol phosphate oxidase	≥ 1000 IU/l
• Peroxidase	≥ 500 IU/l
• ATP	1 mmol/l
• Chromogens	2 mmol/l
• Activators & stabilizers	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Avoid the use of plastic or siliconized container which may prolong clotting time. Serum or plasma should be separated from the cells at the earliest possible (within 30 minutes). For plasma collection following anticoagulants may be used.

• EDTA	2 mg/ml of blood
• CITRATE	6 mg/ml of blood
• HEPARIN	200 IU/ml of blood

Avoid use of Oxalate and Sodium Fluoride as anticoagulant.
Triglycerides are stable for 4 days in neatly separated serum or plasma at 2-8°C.

PROCEDURE

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** ..... **10 mins. at 37°C**
- ☐ **Wavelength** ..... **510 nm. (500-530 nm.)**
- ☐ **Zero setting with** ..... **Reagent Blank**
- ☐ **Blank absorbance limit** ..... **< 0.200 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **200 mg%**
- ☐ **Linearity** ..... **800 mg/dl**

Manual assay procedure

Prewarm at room temperature the required amount of working reagent before use.

Perform the assay as given below :

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.01 ml	0.01 ml	—
Working Reagent	1.0 ml	1.0 ml	1.0 ml

Incubation

Incubate the assay mixture for 10 minutes at 37°C. After incubation, measure the absorbance against blank at 510 nm. (500-530 nm.). Final colour is stable for 30 minutes if not exposed to direct light.

OR

3.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.02 ml	0.02 ml	—
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Mix, incubate for 10 mins. at 37°C.			
Distilled water	2.0 ml	2.0 ml	2.0 ml

Measure the absorbance against blank at 510 nm.or green filter. Linearity of this method strictly depends upon instrument characteristics.

Calculation:

$$\text{Triglycerides in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 200$$

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NOTE :

1. It is important that standard solution is brought to room temperature prior to use or else the result obtained could be erroneous.
2. The specimen to working reagent ratio can be altered proportionally without affecting the results.

EXPECTED VALUES

Upto 170 mg%

Note :
Expected range varies from population to population. It is therefore recommended that each laboratory should establish its own normal range.

PROCEDURE LIMITATIONS

1. Discard the working reagent if the absorbance of the same exceeds 0.200 OD against distilled water at 510 nm.
2. If the triglycerides value exceeds 800 mg% dilute the specimen with saline (1:1 ratio) and repeat the assay. Multiply the result obtained by 2 to get the correct triglycerides value.
3. Glycerol contamination in glassware leads to erroneous results.
4. Care should be taken not to touch reagent and samples with fingers, especially after applying hand lotion which may contain glycerine.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might effect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

1. Foosati, P., et al *Clin. Chem. 28,* 2077 (1982).
2. Henry, J. B., ***Clinical Diagnosis and Management by Laboratory Methods,*** 18th ed., W.B. Saunders, Philadelphia, 1991, p. 204-211.
3. Tietz, N. W., ***Clinical Guide to Laboratory Tests,*** 2nd ed., W.B. Saunders, Philadelphia, 1994, p. 1073 - 1091.
4. Young, D. S., ***Effects of Drugs on Clinical Laboratory Tests,*** 3rd ed., AACC Press, Washington, D.C., 1990, p.3-340 - 3-346.

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE
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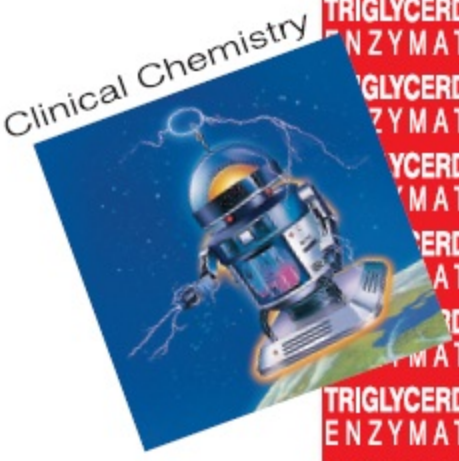
AR. No.: I 21 *TG-2009-05-00I*

ACCUREX BIOMEDICAL PVT. LTD.
Head Office - Mumbai. Tel.: 91(022) 67446744; Fax: 91(022) 66662667
E-mail: accurex@vsnl.com; Website: www.accurex.org
Plant : G-54, MIDC Tarapur, Boisar, Thane - 401 506. INDIA.

Quality Assurance - On line testing



AutoZyme

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