

**L-Type Triglyceride M**

(GPO-HMMPS, Glycerol blanking method)

**For Research Use Only****Not For use in Diagnostic Procedures.**

Code No. 994-02891

990-02991

992-02892

998-02992

**Intended use**

L-Type Triglyceride M is an in vitro assay for the quantitative determination of triglyceride in serum or plasma.

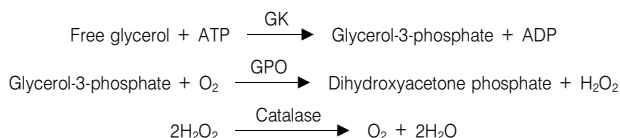
**Summary and explanation of the test**

Lipids in serum or plasma consist of triglyceride, cholesterol, phospholipids, free fatty acids and small amounts of fat-soluble compounds such as fat-soluble vitamins and carotene. Triglycerides are water-insoluble lipids, consisting of fatty acids linked to glycerol.

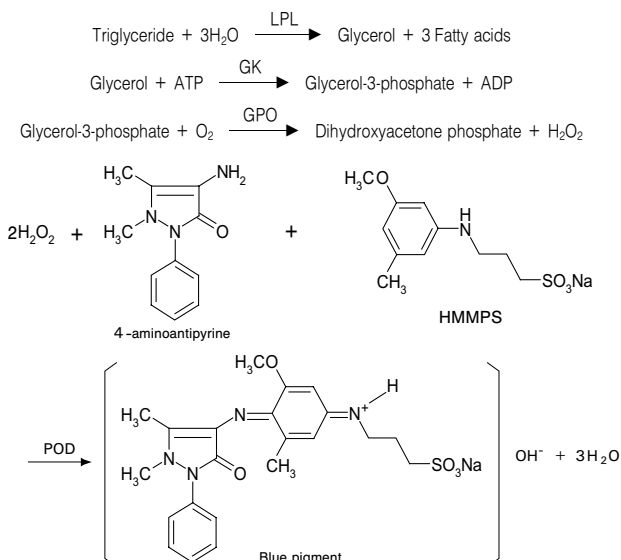
The clinical method using acetylacetone has been traditionally used to measure triglyceride. However, the method requires a complicated procedure such as adsorption or extraction. Therefore, the chemical method has been replaced with the specific methods employing enzymes. The enzymatic method employing glycerol-3-phosphate oxidase (GPO) has been used extensively with growing popularity since it is superior in specificity and simplicity, and the measurement can be done under ordinary conditions. L-Type Triglyceride M is an enzymatic method utilizing N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) that produces blue pigment. This method is not affected by free glycerol in the sample. Before the enzymatic hydrolysis of triglyceride, free glycerol in the sample is decomposed by a series of enzymes that are not involved in the reaction.

**Principle of the method**

When a sample is mixed with Enzyme Color A, free glycerol in the sample is decomposed by glycerol kinase (GK), GPO and catalase contained in the Enzyme Color A, Reagent.



When the Enzyme Color B is added after decomposition of free glycerol, triglycerides in a sample are hydrolyzed to glycerol and free fatty acids in a reaction catalyzed by lipoprotein lipase (LPL). Glycerol is converted to glycerol-3-phosphate by GK in the presence of adenosine-5'-triphosphate (ATP). Glycerol-3-phosphate formed is oxidized by GPO in a reaction that produces hydrogen peroxide. The hydrogen peroxide produced causes HMMPS and 4-aminoantipyrine to undergo a quantitative oxidative condensation catalyzed by peroxidase (POD), producing a blue pigment. The amount of triglycerides contained in the sample is determined by measuring the absorbance of the blue color.

**Reagents**

- (1) Enzyme Color A
  - 50 mmol/L Good's buffer (pH 7.0) containing
    - 50 U/mL glycerol kinase (GK, Microorganism)
    - 8.0 mmol/L adenosine 5'-triphosphate disodium salt (ATP)
    - 5.6 U/mL glycerol-3-phosphate oxidase (GPO, Microorganism)
    - 150 U/mL catalase (Bovine liver)
    - 0.4 mmol/L N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS)
    - 2.0 U/mL ascorbate oxidase (AOD, Microorganism).
  - Store at 2-10°C.
- (2) Enzyme Color B
  - 50 mmol/L Good's buffer (pH 7.1) containing
    - 250 U/mL lipoprotein lipase (LPL Microorganism)
    - 25 U/mL peroxidase (POD, Horseradish)
    - 4.6 mmol/L 4-aminoantipyrine.
  - Store at 2-10°C.

**Warnings and precautions**

1. For Research Use Only. Not for use in Diagnostic procedures.
2. Not to be used internally in humans or animals.
3. Do not use reagents past the expiration date stated on each reagent container label.
4. Do not use the reagents described above for any purpose other than described herein.
5. Do not use the reagents which were frozen. Such reagents may give false results.
6. After opening the reagent, it is not recommended to store it for more than 1 month at 2-10°C. To store the opened reagents, cap the bottle and keep it under the specified conditions.
7. If the reagents come in contact with mouth, eyes, or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
8. Enzyme Color B contains 0.095% sodium azide as a preservative. Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be well flushed with a large amount of water when discarding the reagents.

**Physical or chemical indications of instability**

The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent deterioration.

**Instruments**

The reagent is designed to be used on commercially available automated analyzers such as Hitachi 917s analyzer. Refer to the operating manual for a description of instrument operation, specifications and calibration.

**Specimen collection and preparation<sup>(3)</sup>**

Serum or plasma can be used as a specimen for testing. Ascorbic acid, bilirubin and hemoglobin do not have a significant effect on the assay. Serum triglycerides are stable for 2 days at 20-25°C, 7 days at 4-8°C and 1 year at -20°C.

**Warning/Biohazard**

Since all specimens are potentially infectious, they should be handled at the Biosafety Level 2 as recommended for any potentially infectious body fluid in the USA Centers for Disease Control/USA National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", and in accordance with any other local or national regulations relating to the safe handling of such materials.

**Procedure for Hitachi 917s analyzer****Materials supplied**

Refer to the section entitled "Reagents."

### Materials required but not supplied

Hitachi 917s analyzer  
Quality control material  
Calibrator : Multi Calibrator Lipids  
(Cat. No. : 464-01601)

### Reagent preparation

Reagent 1 Use Enzyme Color A as supplied. Unopened R1 is stable until expiration date printed on the label. Opened R1 can be used for 1 month at 2-10°C.

Reagent 2 Use Enzyme Color B as supplied. Unopened R2 is stable until expiration date printed on the label. Opened R2 can be used for 1 month at 2-10°C.

### Test procedure

Parameter settings (Hitachi 917s)

Temperature : 37°C

Reagent	L-Type Triglyceride M
ANALYZE	
CH TEST/TYPE	TG/Serum/Plasma
ASSAY	2 POINT END-10
POINT	16-34-0-0
WAVELENGTH (Sub/Main)	700/600
SAMPLE VOL. (NORMAL) (Dec.) (INC.)	2.0-0.0-0
DILUENT	(H2O) -0
REAGENT VOL. R1	180-0-( )-0
R2	0-0-( )-0
R3	60-0-( )-0
R4	0-0-( )-0
ABS LIMIT	
PROZONE LIMIT	0 0 LOWER
CELL DET.	DET. 1
<b>CALIBRATION</b>	
CALIB TYPE	LINEAR
POINT	2/2
WEIGHT	0
AUTO CALIBRATION	0
SD LIMIT	999.9
DUPLICATE LIMIT	500
SENSITIVITY LIMIT	0
S1 ABS LIMIT	-3200/3200
<b>RANGE</b>	
TEST #	
UNIT	mg/dL
REPORT NAME	
DATA MODE	On Board
CONTROL INTERVAL	
INST. FACTOR	a=1.0 b=0.0
TECHNICAL LIMIT	
EXPECTED VALUES	
<b>STD CONC.</b>	
	Conc. Pos. Vol. Predil
1	0.0 H <sub>2</sub> O 2.0 0.0
2	*1 *2 2.0 0.0
3	
4	
5	
6	

\*1 : Input the assigned value of the calibrator.

\*2 : Input the position of the calibrator.

### Results

The final results are automatically calculated and printed in concentration. The results are given in mg/dL.

### Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

### Limitations of the procedure

The measurable range of the L-type Triglyceride M method is 1.1 to 2000 mg/dL. If the triglycerides value exceeds 2000 mg/dL, repeat the assay with a 1/1 dilution of the sample with saline. Multiply the results by two to get the final concentration.

### References

- 1 Burtis, C.A. and Ashwood, E.R., Ed. : Tietz Textbook of Clinical Chemistry, 2nd Ed., Saunders, Philadelphia, 1994
- 2 Spayd, R.W., Bruschi, B., et al. : Clin.Chem., 24, 1343-1350 (1978)
- 3 DG Klinische Chemie Mitteilungen 26 (1995) page 5
- 4 AACC press : Handbook of Lipoprotein Testing (1997)

### Ordering information

Code No.	Product	Package
994-02891	L-Type Triglyceride M Enzyme Color A	4 × 60 mL
990-02991	L-Type Triglyceride M Enzyme Color B	4 × 20 mL
992-02892	L-Type Triglyceride M Enzyme Color A	4 × 35 mL
998-02992	L-Type Triglyceride M Enzyme Color B	4 × 13 mL
464-01601	Multi Calibrator Lipids	4 × for 3 mL

Revision date ; Apr. 1, 2018

994-02891F

Manufactured by  
**FUJIFILM Wako Pure Chemical Corporation**

1-2, Doshomachi 3-Chome, Chuo-Ku Osaka 540-8605, Japan  
Tel : +81-6-6203-3749  
Fax : +81-6-6203-1917  
www.wako-chem.co.jp

Distributed by  
**FUJIFILM Wako Diagnostics U.S.A. Corporation**

1025 Terra Bella Ave., Mountain View, CA 94043 U.S.A.  
Tel : 877-714-1924  
Fax : 804-271-0449  
www.wakodiagnosics.com

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