



Triglyceride Microplate Assay Kit

User Manual

Catalog # CAK1086

(Version 1.3D)

Detection and Quantification of Triglyceride (TC) Content in Urine,
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and
Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....2

II. KIT COMPONENTS.....3

III. MATERIALS REQUIRED BUT NOT PROVIDED.....3

IV. SAMPLE PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. CALCULATION.....6

VII. TYPICAL DATA.....7

VIII. TECHNICAL SUPPORT.....7

IX. NOTES.....7

I. INTRODUCTION

Triglyceride (TC), also known as Triacyl triglyceride or Triacyl-glyceride, is the main constituent in vegetable oil and animal fats. Triglycerides play an important role as energy sources and transporters of dietary fat. In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis, heart disease and pancreatitis.

The assay is initiated with the enzymatic hydrolysis of the triglycerides by lipase to produce glycerol and free fatty acids. The glycerol released is subsequently measured by a coupled enzymatic reaction system with a colorimetric readout at 500 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Diluent	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C
Standard (5 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 9 ml Diluent to dissolve before use.

Dye Reagent: add 10 ml Diluent to dissolve before use.

Assay Buffer (not provided): For 100 ml assay buffer, please mix 35 ml Heptane and 65 ml Isopropanol together.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 500 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Heptane
9. Isopropanol

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10,000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Enzyme	90 μ l	90 μ l	90 μ l
Dye Reagent	100 μ l	100 μ l	100 μ l
Cover the plate adhesive strip and put the plate into the oven, 37 °C for 10 minutes, record absorbance measured at 500 nm.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned} \text{TG } (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{TG } (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{TG } (\mu\text{mol}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned} \text{TG } (\mu\text{mol/ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) / V_{\text{Sample}} \\ &= 5 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \end{aligned}$$

C_{Standard} : the concentration of Standard, 5 mmol/L= 5 $\mu\text{mol/ml}$;

C_{Protein} : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

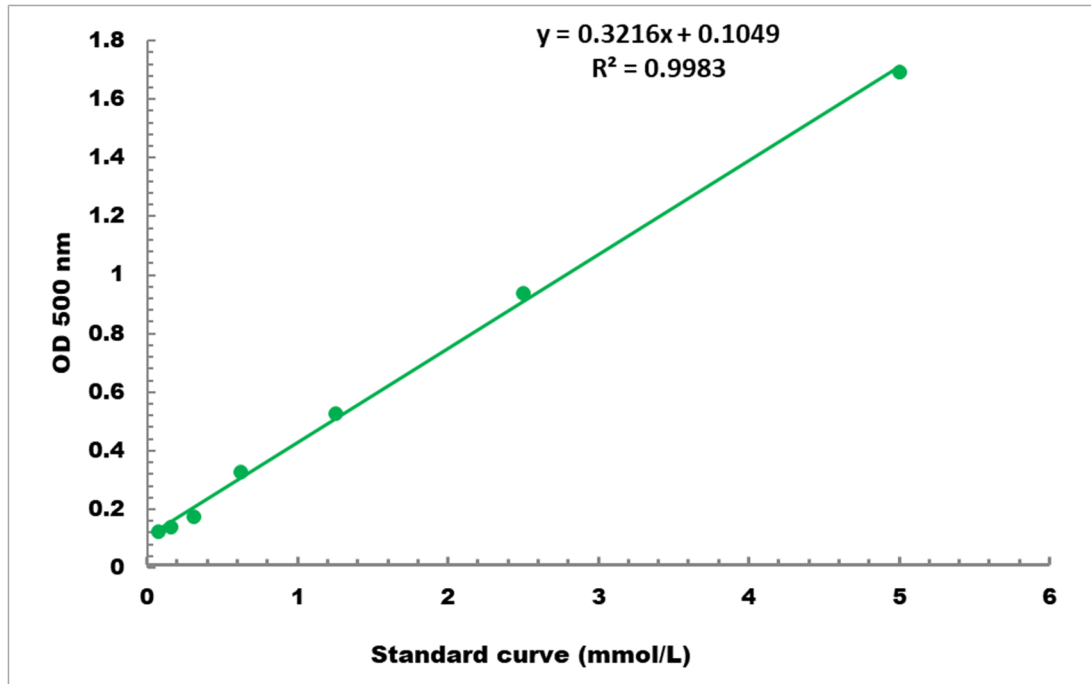
V_{Standard} : the total volume of the reaction, 0.01 ml;

V_{Sample} : the volume of sample, 0.01 ml;

V_{Assay} : the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES