Triglyceride Microplate Assay Kit

Cat#: orb1494226 (User Manual)

I. INTRODUCTION

Triglyceride (TC), also known as Triacyltriglyceride 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
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Heptane
9. Isopropanol

V. 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 × 106 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 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 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	90 µl	90 µl	90 µl
Dye Reagent	100 µl	100 µl	100 µl
Cover the plate adhesive strip and put the plate into the oven, 37 °C for 10 minutes, record absorbance measured at 500 nm.			

VI. CALCULATION

1. According to the protein concentration of sample

TG (mmol /mg) = (CStandard × VStandard) × (ODSample - ODBlank) / (ODStandard - ODBlank) / (VSample × CProtein)
= 5 × (ODSample - ODBlank) / (ODStandard - ODBlank) / CProtein

2. According to the weight of sample
TG (mmol /g) = (CStandard × VStandard) × (ODSample - ODBlank) / (ODStandard - ODBlank) / (W × VSample / VAssay)
= 5 × (ODSample - ODBlank) / (ODStandard - ODBlank) / W

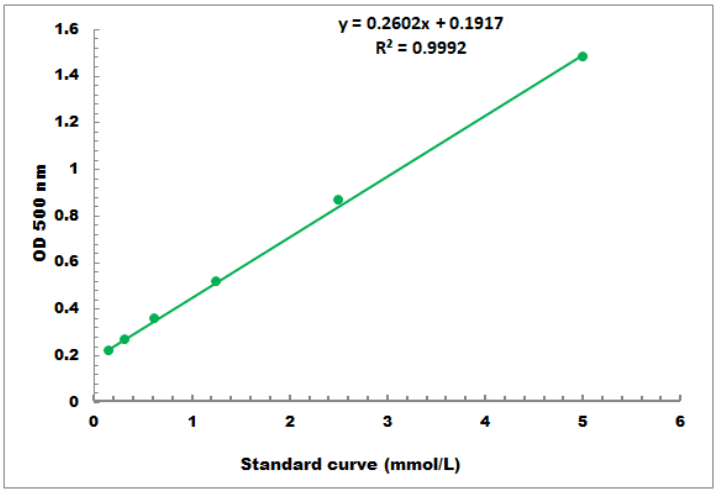
3. According to the quantity of cells or bacteria
TG (mmol /104) = (CStandard × VStandard) × (ODSample - ODBlank) / (ODStandard - ODBlank) / (N × VSample / VAssay)
= 5 × (ODSample - ODBlank) / (ODStandard - ODBlank) / N

4. According to the volume of serum or plasma
TG (mmol /ml) = (CStandard × VStandard) × (ODSample - ODBlank) / (ODStandard - ODBlank) / VSample
= 5 × (ODSample - ODBlank) / (ODStandard - ODBlank)

CStandard: the concentration of Standard, 5 mmol/L;
CProtein: the protein concentration, mg/ml;
W: the weight of sample, g;
N: the quantity of cell or bacteria, N × 104；
VStandard: the total volume of the reaction, 0.01 ml;
VSample: the volume of sample, 0.01 ml;
VAssay: 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

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