Triglyceride

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1086

(Version 1.5F)

Detection and Quantification ofTriglyceride(TC) Contentin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

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.

Triglyceride Colorimetric Microplate Assay Kit 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 550 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Diluent	20 ml x 1	4 °C
Lipase	Powderx 1	-20 °C
Enzyme Mix	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	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Heptane
9. Isopropanol

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.3125/0.156 mmol/L.

Lipase: Add 1 ml Diluent to dissolve before use.Store at -20 °C for 2-3weeks.

Enzyme Mix: Add 8 ml Diluent to dissolve before use.Store at -20 °C for 2-3weeks.

Dye Reagent: Add 10 ml Diluent to dissolve before use.Store at 4°C for 2-3weeksor -20 °C for 2months.

Assay Buffer (not provided): For 100 ml Assay Buffer, please mix 35 ml Heptane and65 ml Isopropanol together.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell samples

Collect cells into centrifuge tube, discard the supernatant after centrifugation, add 0.5mldistilled water for 5 ×10^6 cells, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times). Mix the sample homogenate with 0.5 mlAssay Bufferand vortex thoroughly. Centrifuged at 10,000g 4°C for 10 minutes, then carefully transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 0.5 ml distilled wateron iceadequately.Mix the sample homogenate with 0.5 mlAssay Buffer and vortex thoroughly. Centrifuged at 10,000g 4°C for 10 minutes, then carefully transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directlyordilute samples with Assay Buffer,centrifuged at 10,000g 4°C for 10 minutes, then transfer the upper heptane phase into a new centrifuge tube and keep it on ice for detection.

VI.ASSAY PROCEDURE

If samples do not contain glycerol, add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Lipase	10 µl	10 µl	10 µl
Enzyme Mix	80 µl	80 µl	80 µl
Dye Reagent	100 µl	100 µl	100 µl
Cover the plate adhesive strip and put theplate into the oven,37 °C for 10minutes, record absorbance measured at 550 nm.			

If samples contain glycerol, do a sample background control, omit the Lipase to determine glycerol background, add following reagents intothe microplate:

Reagent*	Sample**	Control	Standard	Blank
Sample	10 µl	10 µl	--	--
Standard	--	--	10 µl	--
Distilled water	--	--	--	10 µl
Diluent	--	10 µl	--	--
Lipase	10 µl	--	10 µl	10 µl
Enzyme Mix	80 µl	80 µl	80 µl	80 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Cover the plate adhesive strip and put theplate into the oven,37 °C for 10minutes, record absorbance measured at 550 nm.				

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII.CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

If samples do not contain glycerol

$$C=\frac{(OD_{Sample}-OD_{Blank})-Intercept}{Slope}\times n(mmol/L)$$

If samples contain glycerol

$$C=\frac{(OD_{Sample}-OD_{Control})-Intercept}{Slope}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

If samples do not contain glycerol

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

If samplescontain glycerol

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^4)$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume Assay Buffer in sample preparation, μl

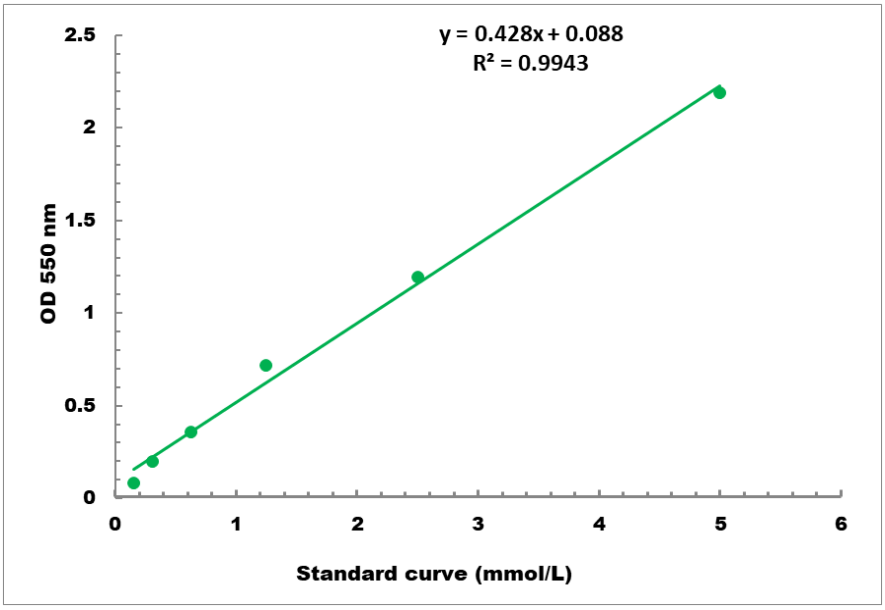
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

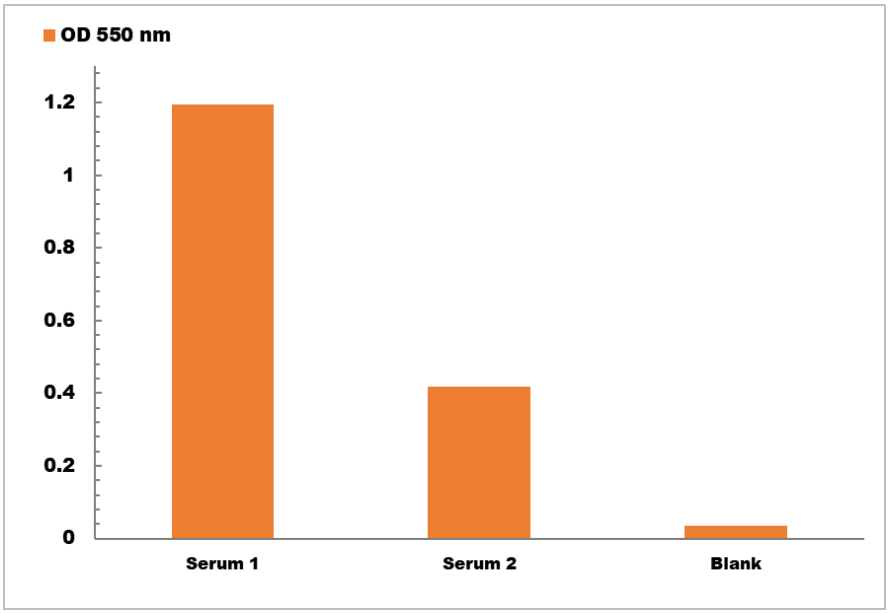
N: the quantity of cell, 10^4

VIII. 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



Determination of triglyceride in serum