



ISO 13485:2016 ISO 9001:2015

Ver. 240703

Triglyceride (TG) Content Assay Kit

BC9907-01 (50 Tests/48 Samples)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Triglyceride (TG) is a fat molecule formed by long-chain fatty acids and glycerol, which is not only the main component of cell membrane, but also an important respiratory substrate. The TG is extracted with isopropyl alcohol, then hydrolysis to glycerol and fatty acids after saponification of TG by KOH. Glycerol is oxidized by periodic acid to form formaldehyde. Condensation of formaldehyde and acetylacetone to form yellow components in presence of chloride ions. The yellow component has a characteristic absorption at 420 nm and proportional to the TG content.

Kit components

Reagent	Volume	Storage
Reagent I	Add 45mL of n-heptane and 45mL of isopropyl alcohol to a glass bottle. Prepare just before use. Not provided.	4°C
Reagent II	7mL × 1	4°C
Reagent III	20mL × 1	4°C
Reagent IV	10mL × 1	4°C
Reagent V	20mL × 1	4°C
Reagent VI	20mL × 1	4°C
Standard	Powder × 1 Add 5mL Reagent I before use to prepare 1mg/mL triglyceride standard solution. Unused standard can be stored at -20°C for 2 weeks. Avoid repeated free- thaw cycles.	4°C

Reagents and Equipment Required but Not Provided

Constant temperature water bath, centrifuge, spectrophotometer, 1mL glass cuvette, n-heptane, isopropyl alcohol and distilled water.

Protocol

I. Sample Preparation

Tissue: Prepare 10% tissue homogenate by adding 1mL Reagent I to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000rpm, 4°C for 10 minutes and collect the supernatant.

Bacteria or cells: Harvest the cells and wash twice with PBS. Add 1mL Reagent I to 5 million cells and ultrasonicate (20% power, work time 2 seconds/ interval 10 seconds repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

Serum or Plasma

Directly use for the assay

II. Assay procedure

- Preheat the spectrophotometer reader/ microplate reader for 30 minutes, adjust wavelength to 420 nm and set zero with distilled water.
- Preheat water bath to 65°C.
- Add the reagents in microcentrifuge tube as mentioned in the table.

Reagent	Blank tube (B)	Standard tube (S)	Test Tube (T)
Standard solution	-	200µL	-
Sample	-	-	200µL
Reagent I	825µL	625µL	625µL
Reagent II	125µL	125µL	125µL

- Mix the tube vigorously for 30 seconds after adding Reagent I, add Reagent II. Allow to stand for several minutes. After layers are formed, 75µL of the upper layer solution is transferred to a new microcentrifuge tube.
- Detecting TG content

Reagent	Blank tube (B)	Standard tube (S)	Test Tube (T)
Upper layer	75µL	75µL	75µL
Reagent III	250µL	250µL	250µL
Reagent IV	75µL	75µL	75µL
Mix thoroughly and heat at 65°C for 3 minutes in a water bath			
Reagent V	250µL	250µL	250µL
Reagent VI	250µL	250µL	250µL
Mix thoroughly and heat at 65°C for 15 minutes in a water bath			

- Cool the mixture to room temperature.
- Measure absorbance at 420nm. A_B , A_T and A_S

Note: Blank and standard needs to be measured only once during the assay

Calculations

1. Serum

$$\begin{aligned} \text{TG (mg/dL)} &= C \times (A_T - A_B) \div (A_S - A_B) \times 100 \\ &= 100 \times (A_T - A_B) \div (A_S - A_B) \end{aligned}$$

2. Tissue

a) Protein concentration

$$\begin{aligned} \text{TG (mg/mg protein)} &= C \times V \times (A_T - A_B) \div (A_S - A_B) \div (C_{pr} \times V) \\ &= (A_T - A_B) \div (A_S - A_B) \div C_{pr} \end{aligned}$$

b) Sample Weight

$$\begin{aligned} \text{TG (mg /g weight)} &= C \times V \times (A_T - A_B) \div (A_S - A_B) \div W \\ &= (A_T - A_B) \div (A_S - A_B) \div W \end{aligned}$$

3. Cell Number

$$\begin{aligned} \text{TG (mg /10}^4 \text{ cells)} &= C \times V \times (A_T - A_B) \div (A_S - A_B) \div D \\ &= (A_T - A_B) \div (A_S - A_B) \div D \end{aligned}$$

V : The volume of Reagent 1, 1mL

C : Standard concentration, 1mg/ mL

100 :1dL=100 mL

C_{pr} : Sample protein concentration (mg/mL)

W : Sample weight(g)

D : Number of bacteria or cell, 10⁴ cell/mL.

Note

1. There are volatile chemicals in this kit. Use appropriate precautions while handling the kit. Do not keep the reagent bottles open for a long time.
2. Mixing and cooling time after addition of the reagents should be uniform for the repeatability of the assay.
3. If the O.D is greater than 1, dilute the sample using Reagent I and repeat the experiment. During calculation of the result, multiply the final outcome with the dilution factor.