Triglyceride (TG) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: BC0625

Size:100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Preservation Condition
Reagent I	Self-supplied reagent	-
Reagent II	Liquid 10 mL×1	2-8℃
Reagent III	Liquid 15 mL×1	2-8℃
Reagent IV	Liquid 5 mL×1	2-8℃
Reagent V	Liquid 15 mL×1	2-8℃
Reagent VI	Liquid 15 mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. Reagent I: Self-supplied reagent, n-heptane and isopropyl alcohol are mixed 1:1 by volume ratio to a glass bottle, which takes about 150mL. Seal and mix well, storage at 2-8℃. The reagent should be prepared just before use.

2. Standard: powder ×1 bottle, add 5 mL of Reagent I before use. 1 mg/mL triglyceride standard solution, the unused reagent can be stored at -20℃ for 2 weeks, avoid repeated freezing and thawing.

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.

Technical Indicators:

Minimum Detection limit:0.0372 mg/mL

Linear Range: 0.0625-3 mg/mL

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/microplate reader, mortar/homogenizer/cell ultrasonic crusher, micro glass

cuvette/96 well plate, vortex oscillators, water bath, adjustable pipette, n-heptane (>98%, AR), isopropyl alcohol (>98%, AR), distilled water, glass bottle.

Operation procedure:

I. Sample preparation:(The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1) Tissue sample:

Add Reagent I according to the ratio of tissue mass (g) : Reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Reagent I), after ice bath homogenization, centrifuge at 4℃, 8000g for 10min, take supernatant and placed on the ice for test.

2) Bacteria or cell:

The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 2 s，interval 1 s，total time 1min). Centrifuge at 4℃, 8000g for 10min, take supernatant and placed on the ice for test.

3) Serum (plasma) sample:

Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure:

1. Preheat the spectrophotometer/ microplate reader for more than 30 minutes, adjust the wavelength to 420 nm, set zero with distilled water.
2. Preheat water bath to 65℃.

Operation table:

Reagent name (μL)	Blank tube (A_B)	Standard tube (A_S)	Test tube (A_T)
Standard solution	-	120	-
TG test solution	-	-	120
Reagent I	495	375	375
Reagent II	75	75	75
Mix thoroughly after adding Reagent I, add Reagent II, shake strongly for 30 s, stand several minutes. After layering, repeat this three times, 30 μL of the upper layer solution is taken and put it			

3. Detect TG content operation table:

Reagent name (μL)	Blank tube (A_B)	Standard tube (A_S)	Test tube (A_T)
Upper layer solution	30	30	30
Reagent III (uL)	100	100	100
Reagent IV (uL)	30	30	30
Mix thoroughly, water bath at 65℃ for 3 minutes. Cool to room temperature			
Reagent V (uL)	100	100	100
Reagent VI (uL)	100	100	100
Mix thoroughly, water bath at 65℃ for 15 minutes. Cool to room temperature			

After cooling, transfer 200μL of liquid from the EP tube to micro glass cuvette/96 well flat-bottom plate, and determine the absorbance at 420 nm. Record them as A_B、A_S and A_T. The blank and standard tubes only need to be measured 1-2 times.

III. Calculation:

1 TG content in serum (plasma)

$TG\ (mg/dL)= C\times(A_T- A_B)\div(A_S- A_B)\times100 =100\times(A_T- A_B)\div(A_S- A_B)$

2 TG content in tissue or bacteria/cell:

(1) Calculate by sample protein concentration:

$TG\ (mg/mg\ prot)= C\times V\times(A_T- A_B)\div(A_S- A_B) \div(Cpr\times V)=(A_T- A_B)\div(A_S- A_B) \div Cpr$

(2) Calculate by sample mass：

$TG\ (mg/g) = C\times V\times(A_T- A_B)\div(A_S- A_B) \div W=(A_T- A_B)\div(A_S- A_B) \div W$

(3) Calculate by the number of bacteria or cells:

$TG\ (mg /10^4\ cell) = C\times(A_T- A_B)\div(A_S- A_B)\times V\div D=(A_T- A_B)\div(A_S- A_B) \div D$

100：Unit conversion factor, 1dL =100 mL;

V: The volume of reagent1, 1 mL;

C: Standard concentration, 1 mg/ mL;

Cpr: Sample protein concentration (mg/mL);

W: Sample weight(g);

D: Density of bacteria or cell, 10^4 cell/mL.

Note:

1. There are volatile substances in the kit. Gloves and masks should be worn during the experiment. The reagent bottle cap should be closed in time after opening.

2. After the addition of Reagent II, it is necessary to repeatedly and violently vibrate, so that the triglyceride in the test solution can be fully extracted, and the oscillation amplitude, time, repeated times and waiting for stratification time should be consistent.

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3. In order to ensure the repeatability of the test, the cooling time after each water bath should be unified.

4. If the OD value of the test tube is greater than 1.5, it is recommended to dilute the sample with Reagent I properly before testing, and multiply it by the corresponding dilution multiple during calculation.

Recent Products Citations:

[1] Ye Q, Liu Y, Zhang G, Deng H, Wang X, Tuo L, Chen C, Pan X, Wu K, Fan J, Pan Q, Wang K, Huang A, Tang N. Deficiency of gluconeogenic enzyme PCK1 promotes metabolic-associated fatty liver disease through PI3K/AKT/PDGF axis activation in male mice. Nat Commun. 2023 Mar 14;14(1):1402. doi: 10.1038/s41467-023-37142-3. PMID: 36918564; PMCID: PMC10015095.

[2] Ning Z, Guo X, Liu X, Lu C, Wang A, Wang X, Wang W, Chen H, Qin W, Liu X, Zhou L, Ma C, Du J, Lin Z, Luo H, Otkur W, Qi H, Chen D, Xia T, Liu J, Tan G, Xu G, Piao HL. USP22 regulates lipidome accumulation by stabilizing PPAR in hepatocellular carcinoma. Nat Commun. 2022 Apr 21;13(1):2187. doi: 10.1038/s41467-022-29846-9. PMID: 35449157; PMCID: PMC9023467.

[3] Gao H, Zhou L, Zhong Y, Ding Z, Lin S, Hou X, Zhou X, Shao J, Yang F, Zou X, Cao H, Xiao G. Kindlin-2 haploinsufficiency protects against fatty liver by targeting Foxo1 in mice. Nat Commun. 2022 Feb 23;13(1):1025. doi: 10.1038/s41467-022-28692-z. PMID: 35197460; PMCID: PMC8866405.

[4] Wei X, Yin F, Wu M, Xie Q, Zhao X, Zhu C, Xie R, Chen C, Liu M, Wang X, Ren R, Kang G, Zhu C, Cong J, Wang H, Wang X. G protein-coupled receptor 35 attenuates nonalcoholic steatohepatitis by reprogramming cholesterol homeostasis in hepatocytes. Acta Pharm Sin B. 2023 Mar;13(3):1128-1144. doi: 10.1016/j.apsb.2022.10.011. Epub 2022 Oct 13. PMID: 36970193; PMCID: PMC10031266.

[5] Teng P, Cui K, Yao S, Fei B, Ling F, Li C, Huang Z. SIRT5-mediated ME2 desuccinylation promotes cancer growth by enhancing mitochondrial respiration. Cell Death Differ. 2024 Jan;31(1):65-77. doi: 10.1038/s41418-023-01240-y. Epub 2023 Nov 25. PMID: 38007551; PMCID: PMC10781994.

References:

[1] Fletcher M J. A colorimetric method for estimating serum triglycerides[J]. Clinica Chimica Acta, 1968, 22(3): 393-397.

[2] Hercules D M, Sheehan T L. Chemiluminescent determination of serum glycerol and triglycerides[J]. Analytical chemistry, 1978, 50(1): 22-25.

Related Products:

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BC0750/BC0755	Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit
BC0410/BC0415	Acetyl CoA carboxylase(ACC) Activity Assay Kit
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