Triglyceride (TG) Colorimetric Assay Kit (Single Reagent, GPO-PAP Method)

Catalog Number EEA028 (96 tests)
Rev 1.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

This kit applies the GPO-PAP method and it can be used for in vitro determination of triglyceride (TG) content in serum, plasma and tissue samples. TG is the main component of vegetable oil, animal fat, low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), and serves as a carrier and source of energy for fatty acids. Triglyceride turnover rate determines the utilization of fatty acids in mammalian tissues. Any dysfunction in this process may lead to changes in glucose metabolism, insulin resistance and type 2 diabetes.

Triglycerides (TG) can be hydrolyzed by lipoprotein lipase into glycerol and free fatty acids. Glycerol produces glycerol-3-phosphate and ADP under the catalysis of glycerol kinase (GK). Glycerol-3-phosphate produces hydrogen peroxide under the action of glycerol phosphate oxidase (GPO). In the presence of 4-aminoantipyrine and phenol, hydrogen peroxide is catalyzed by peroxidase (POD) to produce quinones which is proportional to the content of TG.

Contents and storage

Kit and components are shipped at 2-8℃. An unopened kit can be stored at 2-8℃ for 12 months.

Components	Quantity (96 tests)
Enzyme Working Solution	25 mL
2.26 mmol/L Glycerinum Standard	0.1 mL
Microplate	1 plate
Plate Sealer	2 pieces

Required materials

- Distilled or deionized water
- Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4), Anhydrous ethanol
- Microtiter plate reader with software capable of measurement at or near 510 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Incubator capable of maintaining 37℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum and plasma samples: Detect the sample directly. If the sample is turbid, centrifuge at 10000 g for 10 min at 4℃, then take the supernatant for detection.

Tissue sample:

- Take 0.02-1 g fresh tissue to wash with homogenization medium at 2-8℃ to remove blood cells.
- Absorb the water with filter paper and weigh.
- Homogenize at the ratio of the volume of anhydrous ethanol (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃.
- Take the supernatant and preserve it on ice for detection.

If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for 1 month

Prepare samples

It is recommended to take 2~3 samples with expected large difference to do a pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.14-10 mmol/L).

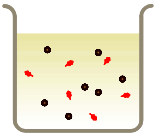
Note: Use all samples with 2 hours of dilution

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat plasma	1
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent of serum (plasma) is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4); The diluent of tissue is anhydrous ethanol.

Assay Procedure

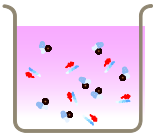


1. Add sample, blank and standard

a. **Blank well:** Take 2.5 μL of distilled or deionized water to the wells.

b. **Standard well**: Take 2.5 μL of 2.26 mmol/L glycerinum standard to the wells.

c. **Sample well**: Take 2.5 μL of sample to the wells.



2. Add substrate

a. Add 250 μL of enzyme working solution into each wells.

b. Incubate at 37 ℃ for 10 min, then measure the OD values of each well with microplate reader at 510 nm.

Calculation

Serum (plasma) and other liquid sample:

$$\underset{(\text{mmol/L})}{\text{TG}} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

Tissue sample:

$$\underset{(\mu\text{mol/g wet weight})}{\text{TG}} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div \frac{m}{V}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard.

f: Dilution factor of sample before test.

m: the weight of tissue sample, g.

V: the volume of anhydrous ethanol, mL.

To easy calculate the test results, refer to the calculation file available on the webpage.

Example analysis

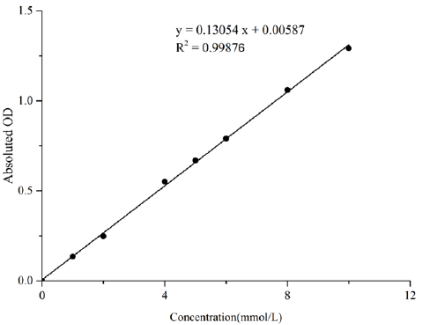
Take 2.5 μL of mouse serum sample and carry the assay with microplate reader according to the operation table. The results are as follows: the average OD value of the sample is 0.314, the average OD value of the standard is 0.407, the average OD value of the blank is 0.080, and the calculation result is:

$$\underset{(\text{mmol/L})}{\text{TG}} = \frac{0.314\text{-}0.080}{0.407\text{-}0.080} \times 2.26 = 1.62 \text{ mmol/L}$$

Performance characteristics

- Standard curve (example)

The following data were obtained for the various standards over the range of 0–10 mmol/L standard.

Concentration (mmol/L)	OD at 510 nm	Standard Curve
10	1.375	
8	1.144	
6	0.874	
5	0.752	
4	0.635	
2	0.332	
1	0.219	
0	0.084	

- Inter-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.50	2.25	9.00
%CV	13.8	8.4	5.5

CV = Coefficient of Variation

- Intra-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.80	4.50	8.50
%CV	5.8	1.8	4.7

CV = Coefficient of Variation

- Expected values

This assay was tested with human serum, and plasma samples without dilutions.

Sample Type	Range (**mmol/L**)	Average (**mmol/L**)
Human serum	0.45-1.69	1.27
Human plasma	0.45-1.69	0.662

- Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Standard 1 (high conc.)	Standard 2 (middle conc.)	Standard 3 (low conc.)
Expected Conc. (mmol/L)	0.8	4.5	8.5
Observed Conc. (mmol/L)	1.20	4.86	9
Recovery rate (%)	115	101	99

- Recommended Plate Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
B	B	B	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
C	S1	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
D	S2	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
E	S3	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
F	S4	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
G	S5	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
H	S6	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92

[Note]: A, blank wells; B, standard wells; S1-S92, sample wells.

- Sensitivity

The analytical sensitivity of the assay is 0.14 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support.**

Corporate entity: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For support visit thermofisher.com/support
or email techsupport@lifetech.com.
thermofisher.com
June 2023

ThermoFisher SCIENTIFIC