PRODUCT INFORMATION & MANUAL

Triglyceride Assay Kit (Fluorometric)

NBP3-24555

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

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Triglyceride Assay Kit (Fluorometric)

Catalog No: NBP3-24555

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.8 μmol/L

Detection range: 0.8-30 μmol/L

Average intra-assay CV (%): 3.9

Average inter-assay CV (%): 9.1

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used for the determination of triglyceride in serum, plasma, tissue and cell samples.

▲Detection principle

Triglyceride is converted by enzyme to produce hydrolytic products, which is catalyzed by enzymes to produce fluorescent substances. The content of triglyceride in samples can be calculated by measuring the fluorescence value.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Extraction Solution	50 mL × 2 vials	2-8°C, 12 months
Reagent 3	Probe	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	1 mmol/L Standard Solution	0.5 mL × 1 vial	2-8°C, 12 months
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Centrifuge, Incubator(37°C)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

The preparation equipment needs to be cleaned several times before the preparation of the chromogenic working solution to prevent contamination by impurities.

Pre-assay preparation

▲Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of chromogenic working solution:
   Mix the reagent 1 and reagent 3 at a ratio of 49:1. Prepare the fresh needed amount before use and the prepared solution should be stored with shading light.
3. The preparation of 30 μmol/L standard solution:
   Mix the reagent 4 and reagent 2 at a ratio of 30:970. The prepared solution can be stored at 2-8°C for 3 days.

▲Reagent preparation

1. Serum and plasma samples: Detect directly.

2. Tissue sample:

Weigh the tissue accurately and add reagent 2 at a ratio of weight (g): volume (mL) =1: 9, homogenize the tissue in ice bath, centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant.

3. Cell sample:

Collect the 1×10^6 cells, add 0.2 mL reagent 2. Homogenize the cells sample with homogenizer on ice. Centrifuge the homogenized cells at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.8 -30 μmol/L).

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

Sample type	Dilution factor
10% Porcine heart tissue homogenate	3-6
10% Porcine liver tissue homogenate	8-10
1×10^6 CHO cells	1-2
1×10^6 293T cells	1-2
Human serum	100-120
Mouse serum	100-120

Note: The diluent is reagent 2.

Assay protocol

▲Plate set up

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

Note:A-H, standard wells; S1-S80, sample wells.

▲ Detailed operation steps

1. The preparation of standard curve:

Dilute 30 μmol/L standard solution with reagent 2 to a serial concentration. The recommended dilution gradient is as follows: 0, 6, 12, 15, 18, 21, 24, 30 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	30 μmol/L standard solution (μL)	Reagent 2 (μL)
A	0	0	200
B	6	40	60
C	12	80	120
D	15	100	100
E	18	120	80
F	21	140	60
G	24	160	20
H	30	200	0

2. The measurement of samples:

1) Standard well: Add 20 μL of standard with different concentrations into the correspinding wells.

   Sample well: Add 20 μL of sample into the wells.

2) Add 200 μL of chromogenic working solution into each well.

3) Mix fully, incubated at 37°C for 5 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Summary operation table

	Standard well	Sample well
Standard with different concentrations (μL)	20	
Sample (μL)		20
Chromogenic working solution (μL)	200	200
Mix fully, incubated at 37°C for 5 min. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.		

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample.

The standard curve is: y= ax + b.

1. For serum (plasma):

$$\text{TG content (mmol/L)} = (\Delta F - b) \div a \times f \div 1000^*$$

2. For tissue and cell sample:

$$\text{TG content (μmol/gprot)} = (\Delta F - b) \div a \div C_{pr} \times f$$

Note:

y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0).

x: The concentration of standards.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$).

C_{pr}: The concentration of protein in sample, gprot/L.

1000*: 1000 μmol =1 mmol.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

Dilute the human serum for 100times and carry the assay according to the operation table. The results are as follows:

standard curve: y = 152.95 x - 74.284, The average fluorescence value of the sample is 2664, the average fluorescence value of the control is 1316. Then the calculation result is:

TG content (mmol/L) = (2664 -1316 + 74.284) ÷ 152.95 × 100 ÷ 1000 = 0.93 mmol/L