PRODUCT INFORMATION & MANUAL

Triglyceride Assay Kit (Colorimetric)

NBP3-24542

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

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

Catalog No: NBP3-24542
Method: Colorimetric method
Specification: 96T **(**Can detect 92 samples without duplication)
Measuring instrument: Microplate reader, Biochemistry analyzer
Detection range: 0-9.04 mmol/L

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit applies the GPO-PAP method and it can be used for *in vitro* determination of triglyceride (TG) content in serum, plasma, cells, culture supernatant and other samples.

Detection principle

$$\text{Glycerin trilaurate} + \text{H20} \xrightarrow{\text{Lipase}} \text{Glycerinum} + \text{Fatty acid}$$

$$\text{Glycerinum} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol} - 3 - \text{phosphoric acid} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphoric acid} + \text{O2} \xrightarrow{\text{Glycerol-3-Phosphate Oxidase}} \text{Hydroxyacetone phosphate} + \text{H2O2}$$

$$\text{Parachlorphenol} + \text{H202} + 4 - \text{AAP} \xrightarrow{\text{Peroxidase}} \text{Red quinone}$$

The color depth of the generated quinones is directly proportional to the triglyceride content. The absorbance values of the standard tube and the sample tube are measured respectively, and the triglyceride content in the sample can be calculated.

Reagent Composition

Composition	Component	Concentration	Size	Storage
Working Solution (Enzyme)	Tris-HCL Buffer	100 mmol/L	25 mL ×1 vial	2-8℃ (shading light)
	Lipase	≥ 3000U/L		
	ATP	0.5 mmol/L		
	Glycerol Kinase	≥ 1000U/L		
	Glycerol-3-Phosphate Oxidase	≥ 5000U/L		
	Peroxidase	≥ 1000U/L		
	4-Aminoantipyrine	1.4 mmol/L		
	Parachlorphenol	3 mmol/L		
2.26 mmol/L Glycerinum Standard			0.1 mL ×1 vial	
96 Well Microplate				Room temperature

Experimental instrument

Test tube, Micropipettor, Vortex mixer, Water bath, Microplate reader or Biochemical analyzer (510 nm)

Sample preparation

1. **Serum (Plasma):** Detect the sample directly. If the concentration is beyond the linear range, then dilute the sample with normal saline before detection.

2. **Culture supernatant sample:** Collect the culture supernatant, centrifuge at 1000 rpm for 10 min, and take the supernatant for detection.
   [Note]: It is generally recommended that the cell density should be more than 1×10^6/mL.

3. **Tissue sample:** Accurately weigh the tissue weight, add 9 times the volume of homogenate media according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 2500 rpm for 10 min, then take the supernatant for detection.
   [Note]: (1) If the tissue sample is not a high-fat sample, the homogenate media should be phosphate buffer (0.1 mol/L, pH 7.4) or normal saline.
   (2) If the tissue sample is high-fat sample or partly high lipid sample, the homogenate media should be absolute alcohol.

4. **Cell sample:**
   Cell collection: Take the prepared cell suspension and centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment. Wash the sediment with isosmotic solution (0.1 mol/L, pH7~7.4 phosphate buffer was recommended) 1~2 times, centrifuge at 1000 rpm for 10 min. Discard the supernatant and keep the cell sediment.
   Cell disruption: Add 0.2~0.3 mL of homogenate media (0.1 mol/L, pH7~7.4 phosphate buffer or normal saline was recommended). Sonicate in ice water bath (power: 300 W, 3~5 second/time, interval for 30 sec, repeat for 3~5 times) or grind with hand-operated. The prepared homogenate kept for detection without centrifugation. The cell can also be lysed with the cell lysate buffer (Triton X-100, 1~2%, 30~40 min), then take the prepared lysate for detection directly without centrifugation.
   [Note]: It is generally recommended that the cell density should be more than 1×10^6/ml. The disrupted cell can be observed with microscope that whether the cell is broken completely.

Operation steps

Operate with 96T microplate. Colorimetric assay by microplate reader			
	Blank well	Standard well	Sample well
Distilled water (μL)	2.5		
Standard (μL)		2.5	
Sample (μL)			2.5
Working solution (μL)	250	250	250

Mix thoroughly, incubate at 37℃ for 10 min, measure the OD value at 510 nm with microplate reader.

Operate with automatic biochemical analyzer	
Sample volume/ Distilled water (μL)	2.5
Working solution (μL)	250
Incubate at 37℃ for 10 min, set zero with distilled water + working solution, measure the absorbance value A at 510 nm.	
Main wavelength (nm)	510
Reaction type	Endpoint method
Reaction direction	(+)

Calculation of results

1. **For serum and other liquid sample:**

   Operated with microplate reader:

   Triglyceride concentration (*mmol/L*)

   $$= \frac{\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}}{\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}} \times \text{Concentration of standard } (mmol/L)$$

   Operated with automatic biochemical analyzer:

   Triglyceride concentration (*mmol/L*)

   $$= \frac{\mathrm{A_{Sample}}}{\mathrm{A_{Standard}}} \times \text{Concentration of standard } (mmol/L)$$

2. **For tissue and cells samples:**

   Operated with microplate reader:

   Triglyceride concentration (*mmol/gprot*)

   $$= \frac{\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}}}{\mathrm{OD_{Standard}} - \mathrm{OD_{Blank}}} \times \text{Concentration of standard } (mmol/L)$$

   ÷ Protein concentration of tested sample (*gprot/L*)

   Operated with automatic biochemical analyzer:

   Triglyceride concentration (*mmol/gprot*)

   $$= \frac{\mathrm{A_{Sample}}}{\mathrm{A_{Standard}}} \times \text{Concentration of standard } (mmol/L)$$

   ÷ Protein concentration of tested sample (*gprot/L*)

Performance index

1. The absorbance of blank tube is ≤ 0.200 (optical path=0.5 cm).
2. **Linear range:** 0~9.04 mmol/L, $r^2 > 0.995$.
3. **Sensitivity:** The absorbance value (ΔA) is between 0.2200-0.2900 when testing 2.26 mmol/L samples.
4. **Accuracy:** Relative deviation ≤ 10%.
5. **Repeatability:** Precision ≤ 5.0%, inter-CV ≤ 8%.
6. **Storage:** The validity of kit is 12 months when stored at 2-8℃ in the dark. It is stable for 1 month when stored at 2-8℃ in the dark after opening.

Notes

1. The kit is for scientific research only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 12 months.
4. Do not use components from different batches of kit.
5. If the sample content is beyond the maximum limit, please dilute the sample with normal saline before detection, and multiply the result by the dilution ratio.
6. Protect the reagent from contamination of glucose, cholesterol, etc.
7. The amount of reagent and sample can be increased and decreased as the ratio of 1:100 according to the requirement of automatic biochemical analyzer.

Appendix: Standard curve

(This is for reference only)