

EZ- Total Cholesterol Assay Kit

Metabolism assay kit
(Colorimetric/Fluorometric)

Cat. No. DG-TSC100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Total Cholesterol Assay kit is a product that can quantitatively analyze free cholesterol and cholesteryl esters individually or as total through colorimetric and fluorometric methods.

Most cholesterol in the blood exists in the form of cholesteryl esters, which are hydrolyzed into cholesterol by cholesterol esterase. Cholesterol is then oxidized by cholesterol oxidase, generating H_2O_2 as a by-product, which reacts with the cholesterol probe to cause color development (maximum absorbance 570 nm) and fluorescence (Ex/Em = 535/590 nm).

In reactions where cholesterol esterase is present, total cholesterol (cholesterol + cholesteryl esters) is analyzed, and in reactions where cholesterol esterase is not present, free cholesterol can be analyzed. Cholesteryl ester can be analyzed by the difference between the total cholesterol analysis value and the free cholesterol value.

▪ Contents and Storage Conditions

| Component | 100 assay | Cap Cord | Storage |
|--------------------------------------|-----------|----------|---------|
| Cholesterol Assay buffer | 25mℓ | - | -20°C |
| Cholesterol Enzyme mix (Lyophilized) | 1 vial | Red | -20°C |
| Cholesterol esterase (Lyophilized) | 1 vial | Blue | -20°C |
| Cholesterol Probe | 200μℓ | Yellow | -20°C |
| Cholesterol Standard (2 μg/μℓ) | 100μℓ | Green | -20°C |

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Sample type

The concentration of cholesterol can be measured in samples such as animal tissue, cultured cells, and serum, and this can be used to analyze lipid metabolism.

▪ Preparation of Reagent

| Component | Preparation | Storage and Stability |
|--------------------------------------|--|--|
| Cholesterol Enzyme mix (Lyophilized) | Add 220 μl Assay Buffer and mix well using a pipette. | The mixed solution is stable for 2 months at -20°C . |
| Cholesterol esterase (Lyophilized) | Add 220 μl Assay Buffer and mix well using a pipette. | The mixed solution is stable for 2 months at -20°C . |
| Cholesterol Probe | Use after sufficiently dissolving at room temperature. | The solution can be stored at -20°C , but it is recommended to use it within 2 months. |

* Assay buffer is used after sufficiently warming up to room temperature before experiment.

* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Sample preparation

Add 2-50 μl of the prepared sample to a 96-well plate, and adjust the final volume to 50 μl with assay buffer. ($n \geq 2$)

1) Serum sample : Use 0.5 to 5 μl of serum diluted 10-fold in cholesterol assay buffer.

2) Cell or Tissue (non-aqueous samples)

① Prepare $\sim 1 \times 10^6$ cells or ~ 10 mg tissue sample.

* If you are testing larger quantities of sample, simply increase the reagents proportionally at each step to match the sample amount.

② Wash the sample using PBS.

③ Add 200 μl of chloroform : isopropanol : NP-40 (7:11:0.1) solution to the sample and homogenize on ice.

④ Centrifuge at 15,000xg for 5-10 minutes.

⑤ Transfer all liquid (organic phase) except the pellet to another tube and dry at 50°C to remove chloroform. Vacuum dry the sample for 30 minutes to remove trace amounts of organic solvent.

⑥ Add 200 μl of cholesterol assay buffer to the dried lipid and mix well by sonication or vortexing until the solution becomes cloudy.

3) For unknown samples or samples being measured for the first time, it is recommended to conduct a preliminary experiment to ensure that the measured values are within the standard curve before use.

- 4) For samples with high background, prepare the same amount of sample used for measurement as a background control.
- 5) Because several compounds in the sample may interfere with the reaction, we recommend correcting the results by spike sample with 2 μg of cholesterol standard for more accurate experiments.

2. Standard preparation

1) Colorimetric method

Mix 20 μl of 2 $\mu\text{g}/\mu\text{l}$ Cholesterol Standard and 140 μl of assay buffer to make a 0.25 $\mu\text{g}/\mu\text{l}$ standard solution. Add 0, 4, 8, 12, 16 20 μl of this to a 96-well plate respectively and adjust the final volume to 50 μl with assay buffer. This will create a standard set of 0, 1, 2, 3, 4, and 5 $\mu\text{g}/\text{well}$ for each plate.

| Standard No. | Volume of 0.25 $\mu\text{g}/\mu\text{l}$ cholesterol Standard | Volume of Assay buffer | Final standard volume in well | Final standard cholesterol Conc. ($\mu\text{g}/\text{well}$) |
|--------------|---|------------------------|-------------------------------|--|
| 1 | 0 μl | 50 μl | 50 μl | 0 |
| 2 | 4 μl | 46 μl | 50 μl | 1 |
| 3 | 8 μl | 42 μl | 50 μl | 2 |
| 4 | 12 μl | 38 μl | 50 μl | 3 |
| 5 | 16 μl | 34 μl | 50 μl | 4 |
| 6 | 20 μl | 30 μl | 50 μl | 5 |

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2) Fluorometric method

Mix 10 μl of 2 $\mu\text{g}/\mu\text{l}$ Cholesterol Standard and 790 μl of assay buffer to make a 0.025 $\mu\text{g}/\mu\text{l}$ standard solution. Dispense 0, 4, 8, 12, 16, and 20 μl of this into a 96-well plate respectively and adjust the final volume to 50 μl with assay buffer. This will create a standard set of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 $\mu\text{g}/\text{well}$ for each plate.

| Standard No. | Volume of 0.025 $\mu\text{g}/\mu\text{l}$ cholesterol Standard | Volume of Assay buffer | Final standard volume in well | Final standard cholesterol Conc. ($\mu\text{g}/\text{well}$) |
|--------------|--|------------------------|-------------------------------|--|
| 1 | 0 μl | 50 μl | 50 μl | 0 |
| 2 | 4 μl | 46 μl | 50 μl | 0.1 |
| 3 | 8 μl | 42 μl | 50 μl | 0.2 |
| 4 | 12 μl | 38 μl | 50 μl | 0.3 |
| 5 | 16 μl | 34 μl | 50 μl | 0.4 |
| 6 | 20 μl | 30 μl | 50 μl | 0.5 |

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

3. Reaction mixture preparation

This is the volume based on 1 assay. Calculate the amount of assay you want to use in the experiment and prepare the reaction mix with enough volume.

1) Colorimetric method : Total Cholesterol

| Components | Reaction mixture | Background mixture |
|--------------------------|------------------|--------------------|
| Cholesterol Assay buffer | 44 $\mu\ell$ | 46 $\mu\ell$ |
| Cholesterol Enzyme mix | 2 $\mu\ell$ | - |
| Cholesterol esterase | 2 $\mu\ell$ | 2 $\mu\ell$ |
| Cholesterol Probe | 2 $\mu\ell$ | 2 $\mu\ell$ |
| Total | 50 $\mu\ell$ | 50 $\mu\ell$ |

* Free Cholesterol Reaction Mix : Esterase hydrolyzes cholesterol esters into free cholesterol. If you want to selectively measure only free cholesterol in your sample, do not add esterase and replace it with 2 $\mu\ell$ of assay buffer. (Esterase must be added to the standard curve well.)

2) Fluorometric method : Total Cholesterol

| Components | Reaction mixture | Background mixture |
|--------------------------|------------------|--------------------|
| Cholesterol Assay buffer | 45.6 $\mu\ell$ | 47.6 $\mu\ell$ |
| Cholesterol Enzyme mix | 2 $\mu\ell$ | - |
| Cholesterol esterase | 2 $\mu\ell$ | 2 $\mu\ell$ |
| Cholesterol Probe | 0.4 $\mu\ell$ | 0.4 $\mu\ell$ |
| Total | 50 $\mu\ell$ | 50 $\mu\ell$ |

* Free Cholesterol Reaction Mix : Esterase hydrolyzes cholesterol esters into free cholesterol. If you want to selectively measure only free cholesterol in your sample, do not add esterase and replace it with 2 $\mu\ell$ of assay buffer. (Esterase must be added to the standard curve well.)

4. Add 50 $\mu\ell$ of the reaction mixture to each well containing the cholesterol standard and sample using a multi pipette and mix well.

* If you have prepared a background control, add the background mixture to the background control well.

5. After incubation the plate at 37°C with light blocked for 30 minutes, gently shake it and measure it with a microplate reader.

1) Colorimetric : 570 nm

2) Fluorometric : (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

1. Subtract the standard 1 value (blank) from all measurements.
 2. Average duplicate measurements from each standard well and sample well.
 3. Determine the standard curve using the cholesterol standard absorbance.
(Cholesterol standard vs OD_{570nm})
 4. The amount of cholesterol in the sample is calculated by substituting the sample measurement values into the standard curve.
- * If background control is set, the amount of cholesterol is calculated by subtracting the background control measurement value from the sample measurement value.
5. Based on the amount of cholesterol in the sample calculated in 4, the concentration of cholesterol in the sample is calculated using the following formula.

$$\text{Cholesterol concentration of sample (C)} = B/V \times D \text{ (}\mu\text{g}/\mu\text{L)}$$

B : Amount of cholesterol in the measurement well obtained from the standard curve (μg)

V : Amount of sample in a well (μL)

D : Sample dilution ratio (if diluted twice, calculate as x2, not x1/2)

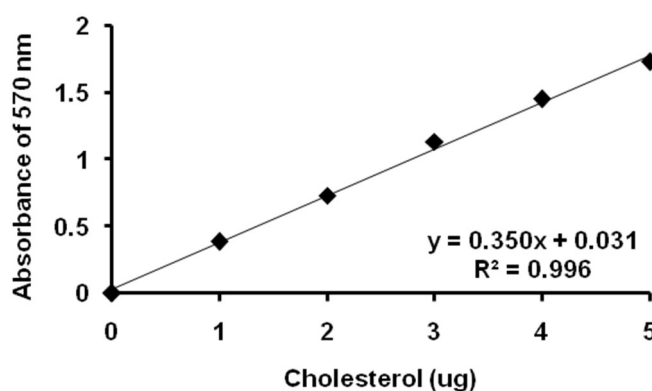
* Cholesterol Molecular Weight: 386.6; 1 $\mu\text{g}/\mu\text{L}$ = 100 mg/dL.

Total Cholesterol (Free cholesterol + Cholesteryl ester)

: Measurements using Total Cholesterol Reaction Mix

Free Cholesterol : Measurements using Free Cholesterol Reaction Mix

Cholesteryl Ester : Total Cholesterol Measurements – Free Cholesterol Measurements



Cholesterol standard curve. Assay was performed following the kit protocol.

※ Spike sample : If there is a possibility that some component in the sample may have affected the reaction, for example, there may be cases where the result shows that only 1.6 ng (80%) of cholesterol is present when in fact 2 ng is present due to the influence of some other substance. To correct for this phenomenon, a separate well is set up with a certain amount of cholesterol (using a standard substance) added to the sample, and the concentration of the actual sample is corrected through the result value. If a spike sample was used in this experiment, the above concentration calculation formula is summarized as follows.

$$\text{Cholesterol in the sample (B)} = \text{OD1} / (\text{OD2} - \text{OD3}) \times \text{Cholesterol spike } (\mu\text{g})$$

OD1 : OD value of Sample (blank corrected)

OD2 : OD value of Spiked sample (blank corrected)

OD3 : OD value of Sample (blank corrected)

Cholesterol spike : Amount of Cholesterol spike added to the sample

▪ Related Product

| | Products | Catalog No. | Assay |
|-----------------------------------|---|-------------|-----------|
| Oxidative Stress Assay Kit | EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric) | DG-SOD400 | 400 Assay |
| | EZ-Glutathione Assay Kit (Colorimetric) | DG-GLU200 | 200 Assay |
| | EZ-Catalase Assay Kit (Fluorometric/Colorimetric) | DG-CAT400 | 400 Assay |
| | EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric) | DG-PER500 | 500 Assay |
| | EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric) | DG-TBA200 | 200 Assay |
| | EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric) | DG-TAC200 | 200 Assay |
| | EZ-DPPH Antioxidant Assay Kit (Colorimetric) | DG-DPH400 | 400 Assay |
| | EZ-ABTS Antioxidant Assay Kit (Colorimetric) | DG-ABT400 | 400 Assay |
| | EZ-Glutathione Peroxidase Assay Kit (Colorimetric) | DG-GPX100 | 100 Assay |
| Metabolism Assay Kit | EZ-Lactate Assay Kit (Colorimetric) | DG-LAC100 | 100 Assay |
| | EZ-Acetylcholinesterase Assay Kit (Colorimetric) | DG-ACE100 | 100 Assay |
| | EZ-Ascorbic Acid Assay Kit (Colorimetric) | DG-ASC100 | 100 Assay |
| | EZ-ATP Assay Kit (Fluorometric/Colorimetric) | DG-ATP100 | 100 Assay |
| | EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric) | DG-FFA100 | 100 Assay |
| | EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric) | DG-FGC100 | 100 Assay |
| | EZ-Glucose Assay Kit (Fluorometric/Colorimetric) | DG-GCS100 | 100 Assay |
| | EZ-HDL, LDL/VLDL Assay Kit (Fluorometric/Colorimetric) | DG-CHO100 | 100 Assay |
| | EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric) | DG-TSC100 | 100 Assay |
| | EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric) | DG-TGC100 | 100 Assay |
| | EZ-Nitric Oxide Assay kit (Colorimetric) | DG-NO500 | 500 Assay |
| | EZ-Total Collagen Assay Kit (Colorimetric) | DG-COL100 | 100 Assay |
| | EZ-Ethanol Assay Kit (Colorimetric) | DG-ETH100 | 100 Assay |