EZ-HDL, LDL/VLDL Assay Kit

Metabolism assay kit

(Colorimetric/Fluorometric)

Cat. No. DG-CHO100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

HDL (High-Density Lipoprotein) cholesterol and LDL (Low-Density Lipoprotein) cholesterol play a central role in a variety of diseases. Low levels of HDL and high levels of LDL are well known to be associated with increased cardiovascular risk.

EZ-HDL, LDL/VLDL Assay Kit provides a simple method to isolate HDL from LDL and VLDL (Very Low-Density Lipoprotein) in serum samples and then quantify HDL and LDL/VLDL.

In the assay, cholesterol oxidase specifically recognizes free cholesterol and reacts with a probe, producing absorbance (λ = 570 nm) and fluorescence (Ex/Em = 538/590 nm). Cholesterol esterase hydrolyzes cholesteryl ester into free cholesterol, so cholesterol ester and free cholesterol can be detected separately in the presence and absence of cholesterol esterase.

▪ Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage
Cholesterol Assay buffer	25㎖	-	-20℃
2X LDL/VLDL buffer	10㎖		
Cholesterol Enzyme mix (Lyophilized)	1 vial	Red	-20℃
Cholesterol esterase (Lyophilized)	1 vial	Blue	-20℃
Cholesterol Probe	200㎕	Yellow	-20℃
Cholesterol Standard (2 ㎍/㎕)	100㎕	Green	-20℃

* 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.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Cholesterol Enzyme mix (Lyophilized)	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
Cholesterol esterase (Lyophilized)	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
Cholesterol Probe	Use after sufficiently dissolving at room temperature.	The solution can be stored at -20℃, 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. Separation of HDL and LDL/VLDL

① Add 100 ㎕ of 2X LDL/VLDL buffer and 100 ㎕ of serum sample to a microtube and mix.

② Incubate at room temperature for 10 minutes.

③ Centrifuge at 2000xg for 10 minutes.

④ The supernatant from the centrifuge is HDL, and the sediment is LDL/VLDL.

* Note: If the supernatant is opaque after centrifugation, centrifuge again and use.
If the supernatant is still opaque, dilute the sample 1:1 with PBS and repeat the above process.

⑤ The supernatant is transferred to a new tube and used for measuring HDL.

⑥ If you want to measure LDL/VLDL, centrifuge the tube again after removing the supernatant, carefully remove as much supernatant as possible, and then dissolve the precipitate in 200 ㎕ PBS and use.

2. Sample preparation

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

1) 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.

2) For samples with high background, prepare the same amount of sample used for measurement as a background control.

3) Because several compounds in the sample may interfere with the reaction, we recommend correcting the results by spiked sample with 2 μg of cholesterol standard for more accurate experiment.

3. Standard preparation

1) Colorimetric method

Mix 20 μℓ of 2 μg/μℓ Cholesterol Standard and 140 μℓ of assay buffer to make a 0.25 μg/μℓ 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 μg/well for each plate.

Standard No.	Volume of 0.25μg/μℓ cholesterol Standard	Volume of Assay buffer	Final standard volume in well	Final standard cholesterol Conc. (μg/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 μg/μℓ Cholesterol Standard and 790 μℓ of assay buffer to make a 0.025 μg/μℓ 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 μg/well for each plate.

Standard No.	Volume of 0.025 μg/μℓ cholesterol Standard	Volume of Assay buffer	Final standard volume in well	Final standard cholesterol Conc. (μg/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.

T. 02-2081-1526 F. 02-855-1550 www.dogenbio.com

4. 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 ㎕	46 ㎕
Cholesterol Enzyme mix	2 ㎕	-
Cholesterol esterase	2 ㎕	2 ㎕
Cholesterol Probe	2 ㎕	2 ㎕
Total	50 ㎕	50 ㎕

* 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 ㎕ 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 ㎕	47.6 ㎕
Cholesterol Enzyme mix	2 ㎕	-
Cholesterol esterase	2 ㎕	2 ㎕
Cholesterol Probe	0.4 ㎕	0.4 ㎕
Total	50 ㎕	50 ㎕

* 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 ㎕ of assay buffer. (Esterase must be added to the standard curve well.)

5. Add 50 ㎕ 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.

6. After incubation the plate at 37℃ 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.

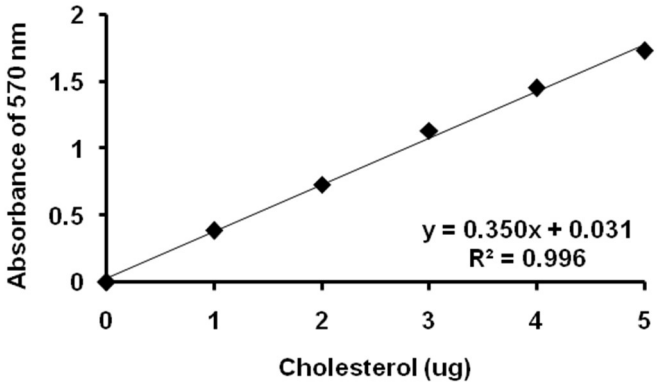
5. Based on the amount of cholesterol in the sample calculated in 4, the concentration of cholesterol in the sample is calculated using the following formula.

Cholesterol concentration of sample (C) = B/V x D (㎍/㎕)

B : Amount of cholesterol in the measurement well obtained from the standard curve (㎍)
V : Amount of sample in a well (㎕)
D : Sample dilution ratio (if diluted twice, calculate as x2, not x1/2)

* Cholesterol Molecular Weight: 386.6; 1 μg/μl = 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.

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※ 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.

Cholesterol in the sample (B) = OD1 / (OD2 – OD3) x Cholesterol spike (μg)

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/VDL 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