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ab65390 HDL and LDL/VLDL Cholesterol Assay Kit (Colorimetric/ Fluorometric)

For the rapid, sensitive and accurate measurement of HDL and LDL/VLDL cholesterol in serum and tissue lysates.

[View kit datasheet: www.abcam.com/ab65390](http://www.abcam.com/ab65390)
(use www.abcam.cn/ab65390 for China, or www.abcam.co.jp/ab65390 for Japan)

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

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1. Overview

HDL and LDL/VLDL Cholesterol Assay Kit (Colorimetric/Fluorometric) (ab65390) provides a simple quantification method of HDL and LDL/VLDL after convenient separation of the different lipoprotein fractions [HDL from LDL and VLDL (very low-density lipoprotein)] in serum and tissue lysate samples. In this assay, cholesterol oxidase specifically recognizes free cholesterol and produces a component that will react with a probe to generate color (OD570 nm) and fluorescence (Ex/Em = 538/587 nm). Because the cholesterol esterase is able to hydrolyze cholesteryl ester into free cholesterol, both cholesterol ester and free cholesterol can be detected separately, depending on the presence or absence of cholesterol esterase in the reactions.

This assay can detect up to 0.1 μg or 1 μg cholesterol when using fluorometric or colorimetric detection, respectively.

Regulation of HDL (high-density-lipoprotein)-cholesterol and LDL (low-density-lipoprotein)-cholesterol plays a central role in various disease developments. It is well known that low levels of HDL and high level of LDL are associated with an increased risk of cardiovascular events.

2. Protocol Summary

Standard curve preparation



Sample preparation + separation HDL and LDL/VLDL



Add Total Cholesterol reaction mix or Free Cholesterol Reaction Mix



Incubate at 37°C for 60 minutes



Measure optical density (OD₅₇₀ nm) or
fluorescence (Ex/Em = 535/587 nm)

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cholesterol Assay Buffer	25 mL	-20°C	-20°C
2X LDL/VLDL Precipitation Buffer	10 mL	-20°C	-20°C
Cholesterol Standard (2 µg/µL)	100 µL	-20°C	-20°C
Cholesterol Probe	200 µL	-20°C	-20°C
Cholesterol Esterase (lyophilized)	1 vial	-20°C	-20°C
Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- Double distilled water (ddH₂O)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce homogenizer (if using tissue)

8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Cholesterol Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 2X Precipitation Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 Cholesterol Standard (2 µg/µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 Cholesterol Probe (in DMSO):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.5 Cholesterol Esterase (lyophilized):

Dissolve in 220 µL Cholesterol Assay Buffer. Keep on ice during the assay. Aliquot esterase so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.6 Enzyme Mix (lyophilized):

Dissolve in 220 µL Cholesterol Assay Buffer. Keep on ice during the assay. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Colorimetric assay:

- 10.1.1 Prepare 200 μL 0.25 $\mu\text{g}/\mu\text{L}$ Cholesterol standard by diluting 25 μL of the provided Cholesterol Standard (2 $\mu\text{g}/\mu\text{L}$ solution) with 175 μL of Cholesterol Assay Buffer.
- 10.1.2 Using 0.25 $\mu\text{g}/\mu\text{L}$ Cholesterol working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Cholesterol 0.25 $\mu\text{g}/\mu\text{L}$ Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End amount Cholesterol in well ($\mu\text{g}/\text{well}$)
1	0	150	50	0
2	12	138	50	1
3	24	126	50	2
4	36	114	50	3
5	48	102	50	4
6	60	90	50	5

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 μL).

10.2 Fluorometric assay:

Δ Note: Detection sensitivity of fluorometric assay is 10 fold higher than colorimetric assay.

- 10.2.1 Prepare a 0.25 $\mu\text{g}/\mu\text{L}$ Cholesterol standard by diluting 5 μL of the provided Cholesterol Standard (2 $\mu\text{g}/\mu\text{L}$ solution) with 35 μL of Cholesterol Assay Buffer.
- 10.2.2 Prepare 250 μL of 0.025 $\mu\text{g}/\mu\text{L}$ Cholesterol standard by diluting 25 μL of 0.25 $\mu\text{g}/\mu\text{L}$ Cholesterol standard with 225 μL of Cholesterol Buffer.
- 10.2.3 Using 0.025 $\mu\text{g}/\mu\text{L}$ (25 $\text{ng}/\mu\text{L}$) Cholesterol working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Cholesterol 25 $\text{ng}/\mu\text{L}$ Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End amount Cholesterol in well ($\mu\text{g}/\text{well}$)
1	0	150	50	0
2	12	138	50	0.1
3	24	126	50	0.2
4	36	114	50	0.3
5	48	102	50	0.4
6	60	90	50	0.5

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 μL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Plasma and Serum (and other biological fluids):

Do not use EDTA when collecting plasma or serum. We recommend using heparin for collection.

11.1.1 Quantification of **Total Cholesterol**:

11.1.1.1 Use samples directly; no preparation step is required. Proceed to Assay Procedure Section (Section 12).

11.1.2 Separation of HDL and LDL/VLDL:

11.1.2.2 Mix 100 μ L sample with 100 μ L 2X Precipitation Buffer in microcentrifuge tubes.

Δ Note: You can use < 100 μ L sample if you don't have enough sample. Simply add the same volume of 2X Precipitation Buffer to your sample and proceed with the sample preparation process.

11.1.2.3 Incubate 10 minutes at room temperature.

11.1.2.4 Centrifuge sample for 10 minutes at RT at 2000 $\times g$ (5,000 rpm on a bench microcentrifuge).

11.1.2.5 Transfer supernatant into a new tube. This is the **HDL fraction**.

Δ Note: If the supernatant is cloudy, sample should be re-centrifuged. If the sample remains cloudy, dilute the sample 1:1 with PBS and repeat the separation procedure from Step 11.1.2.2.

11.1.2.6 Centrifuge the precipitate once more for 10 minutes at RT at 2000 $\times g$ to remove any HDL left in the sample.

11.1.2.7 Remove trace amount of supernatant carefully.

11.1.2.8 Resuspend precipitate in 200 μ L PBS. This is the **LDL/VLDL fraction**.

11.2 Tissue lysates:

Note: this kit is primarily designed for use with serum and plasma samples. It has been successfully used with liver samples (including in Chang et al. 2019, Yu et al. 2018, and Sadh et al. 2017); sample preparation in liver (and other tissue) samples may need optimization and modification by the user for successful use.

11.2.1 Harvest tissue (initial recommendation: 10 mg).

11.2.2 Wash tissue in cold PBS to remove any trace of blood.

11.2.3 Cut in small pieces and homogenize tissue in 100 μL of Cholesterol Assay Buffer with a Dounce homogenizer or pestle sitting on ice, with 10-15 passes.

11.2.4 Centrifuge sample for 5-10 minutes at 4°C at 13,000 $\times g$ using a cold microcentrifuge to remove any insoluble material.

11.2.5 Collect supernatant and transfer to a new tube. This fraction can be used to measure **Total Cholesterol**.

11.2.6 Separation of HDL and LDL/VLDL:

11.2.6.1 Mix 100 μL sample with 100 μL 2X Precipitation Buffer in microcentrifuge tubes.

Δ Note: You can use < 100 μL sample if you don't have enough sample. Simply add the same volume of 2X Precipitation Buffer to your sample and proceed with the sample preparation process.

11.2.6.2 Incubate 10 minutes at room temperature.

11.2.6.3 Centrifuge sample for 10 minutes at RT at 2000 $\times g$ (5,000 rpm on a bench microcentrifuge).

11.2.6.4 Transfer supernatant into a new tube. This is the **HDL fraction**.

Δ Note: If the supernatant is cloudy, sample should be re-centrifuged. If the sample remains cloudy, dilute the sample 1:1 with PBS and repeat the separation procedure from Step 11.1.2.2.

11.2.6.5 Centrifuge the precipitate once more for 10 minutes at RT at 2000 $\times g$ to remove any HDL left in the sample.

11.2.6.6 Remove trace amount of supernatant carefully.

11.2.6.7 Resuspend precipitate in 200 μL PBS. This is the **LDL/VLDL fraction**.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure – Colorimetric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Plate Loading:

- Standard wells = 50 μ L standard dilutions.
- Sample wells for TOTAL cholesterol = 2-50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer).

Δ Note: For plasma and serum we recommend using 1-20 μ L.

- Sample wells for FREE cholesterol = 2-50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer).

Δ Note: For plasma and serum we recommend using 1-20 μ L.

12.2 Cholesterol reaction mix:

- 12.2.1 Prepare 50 μ L of Total Cholesterol Reaction Mix and 50 μ L Free Cholesterol Reaction Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Total Cholesterol Reaction Mix (μ L)	Free Cholesterol Reaction Mix (μ L)
Cholesterol Assay Buffer	44	46
Cholesterol Probe	2	2
Enzyme Mix	2	2
Cholesterol Esterase	2	0

Δ Note: Cholesterol esterase hydrolyzes cholesteryl ester to free cholesterol. If no esterase is added to the reaction, the assay detects only free cholesterol. With the addition of cholesterol esterase, the assay detects total cholesterol (cholesterol and cholesteryl esters).

12.2.2 Add 50 μ L of Total Cholesterol Reaction Mix into each standard and Total Cholesterol sample wells.

12.2.3 Add 50 μ L of Free Cholesterol Reaction Mix into the Free Cholesterol sample wells.

12.3 Measurement:

12.3.1 Mix and incubate at 37°C for 60 min protected from light.

12.3.2 Measure output immediately on a microplate reader at OD 570 nm.

13. Assay Procedure – Fluorometric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.
- For fluorometric assay, using 5x less probe decreases background reading and increases detection sensitivity.

13.1 Plate Loading:

- Standard wells = 50 μ L standard dilutions.
- Sample wells for TOTAL cholesterol = 2-50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer).

Δ Note: For plasma and serum we recommend using 1-20 μ L.

- Sample wells for FREE cholesterol = 2-50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer).

Δ Note: For plasma and serum we recommend using 1-20 μ L.

13.2 Cholesterol reaction mix:

- 13.2.1 Prepare 50 μ L of Total Cholesterol Reaction Mix and 50 μ L Free Cholesterol Reaction Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Total Cholesterol Reaction Mix (μ L)	Free Cholesterol Reaction Mix (μ L)
Cholesterol Assay Buffer	45.6	47.6
Cholesterol Probe	0.4	0.4
Enzyme Mix	2	2
Cholesterol Esterase	2	0

Δ Note: Cholesterol esterase hydrolyzes cholesteryl ester to free cholesterol. If no esterase is added to the reaction, the assay detects only free cholesterol. With the addition of cholesterol esterase, the assay detects total cholesterol (cholesterol and cholesteryl esters).

13.2.2 Add 50 μ L of Total Cholesterol Reaction Mix into each standard and Total Cholesterol sample wells.

13.2.3 Add 50 μ L of Free Cholesterol Reaction Mix into the Free Cholesterol sample wells.

13.3 Measurement:

13.3.1 Mix and incubate at 37°C for 60 min protected from light.

13.3.2 Measure output immediately on a microplate reader at Ex/Em = 535/587 nm.

14. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

14.1 Standard curve calculation:

- 14.1.1 Subtract the mean absorbance/fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance/fluorescence.
- 14.1.2 Average the duplicate reading for each standard and sample.
- 14.1.3 Plot the corrected standard curve reading (absorbance/fluorescence values) and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

14.2 Measure of Cholesterol in the sample:

- 14.2.1 Apply the corrected sample absorbance/fluorescence reading to the standard curve to get Cholesterol (A) amount in the sample wells.
- 14.2.2 Concentration of Cholesterol ($\mu\text{g}/\mu\text{L}$) in the test samples is calculated as:

$$\text{Cholesterol concentration} = \left(\frac{A}{V} * D\right) * DF$$

Where:

A = amount of cholesterol in the sample well calculated from standard curve (μg).

V = sample volume added in the sample wells (μL).

D = sample dilution factor: for total cholesterol = 1; for HDL and LDL/VLDL fractions = 2 (addition of 2X Precipitation Buffer).

DF = additional dilution factor if sample has been diluted further to fit within standard curve range.

14.3 Total Cholesterol, Free Cholesterol and Cholesteryl Ester calculation:

14.3.1 Calculate Total Cholesterol, Free Cholesterol and Cholesteryl esters present in the sample as follows:

Total Cholesterol = Total Cholesterol Sample value

Free Cholesterol = Free Cholesterol Sample value

Cholesteryl esters = Total Cholesterol – Free Cholesterol

Cholesterol Molecular Weight: 386.6 g/mol

1 $\mu\text{g}/\mu\text{L}$ = 100 mg/dL.

15. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

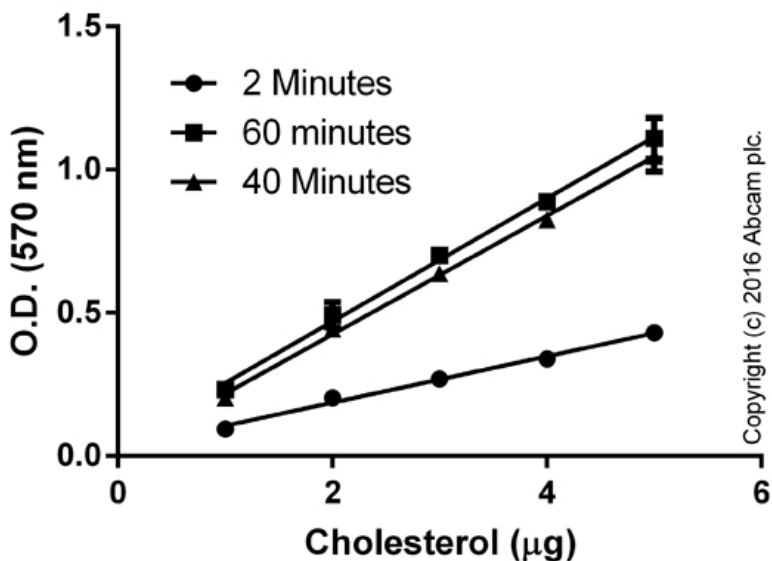
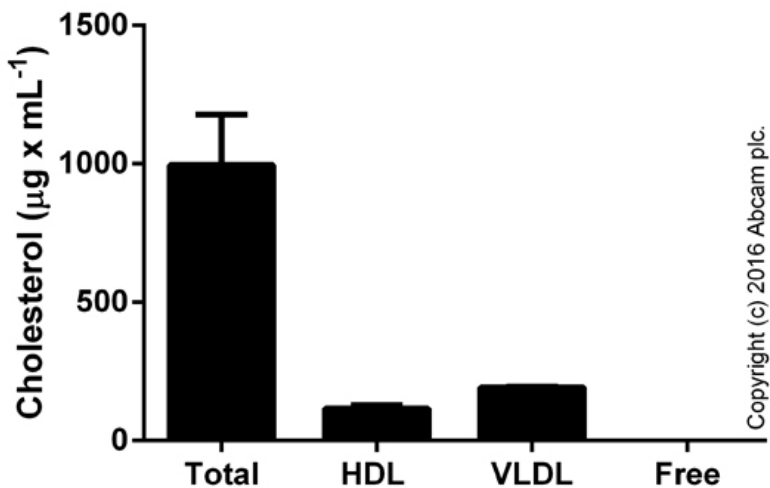


Figure 1. Typical Cholesterol standard calibration curve using colorimetric reading. Signal was measured over a period of time. Background signal (Standard #1) has been subtracted, and each point on the curve represents duplicate values.



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Figure 2. Quantitation of cholesterol in rat serum samples using colorimetric reading. Total (dilution range 1:10-1:100), HDL (dilution range 1:1-1:10), LDL/VLDL (dilution range 1:1-1:10) and free cholesterol (not diluted) was measured in duplicate, after 40 minutes incubation.

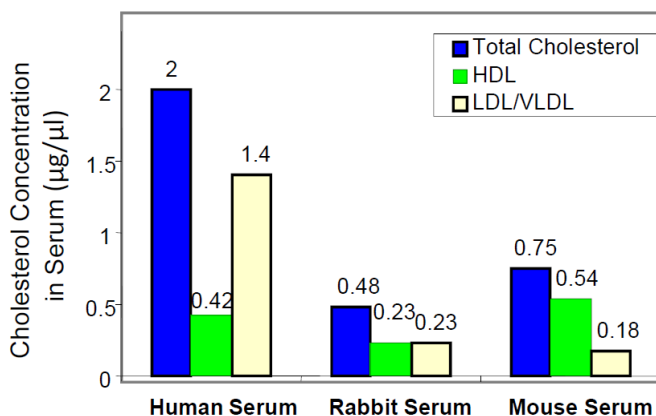


Figure 3. Measurement of total cholesterol, HDL, LDL/VLDL from serum samples from various species. Total Cholesterol (blue bar), HDL (green bar), and LDL/VLDL (yellow bar) cholesterol.

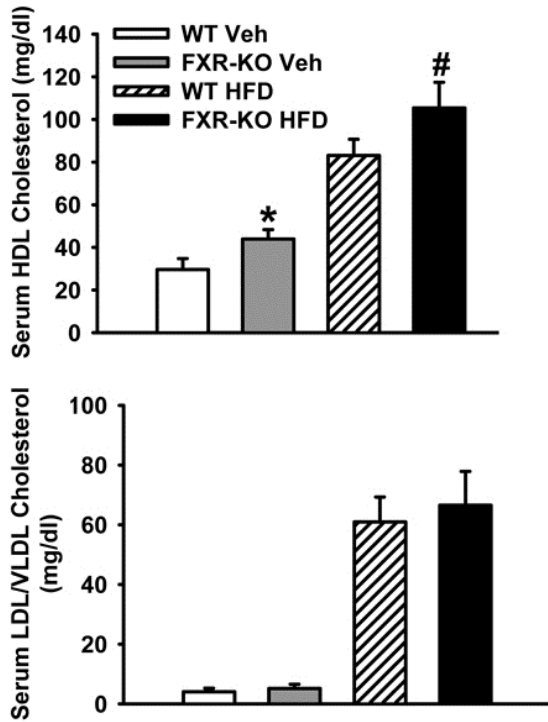


Figure 4: Wild type (WT) or farnesoid X receptor knock-out (FRX-KO) mice were treated with either vehicle (Veh) or a high fat-containing diet (HFD) for 16 weeks. Serum HDL (top) and serum LDL/VLDL cholesterol (bottom) from 5-7 mice/group was measured using ab65390 following protocol instructions. An asterisk (*) means $P < 0.05$ between WT and FXR-KO vehicle group. A pound (#) indicates $P < 0.05$ between WT and FXR-KO HFD group. *Image obtained from Li G et al., PLoS One, 2012; 7(4): e35895*

16. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare Cholesterol standard dilution for your desired detection method: colorimetric [1 – 5 µg/well] or fluorometric [0.1 – 0.5 µg/well].
- Prepare samples (including deproteinization step) in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), total cholesterol samples (50 µL) and free cholesterol sample wells (50 µL).
- Prepare Total Cholesterol Reaction Mix and Free Cholesterol Reaction Mix for colorimetric or fluorometric reading:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
	TOTAL/FREE	TOTAL/FREE
Cholesterol Assay Buffer	44 / 46	45.6 / 47.6
Cholesterol Probe	2 / 2	0.4 / 0.4
Enzyme Mix	2 / 2	2 / 2
Cholesterol Esterase	2 / 0	2 / 0

- Add 50 µL Total Cholesterol Reaction Mix to standard and Total cholesterol sample wells.
- Add 50 µL Free Cholesterol Reaction Mix to Free cholesterol sample wells.
- Incubate plate at 37°C for 60 minutes protected from light.
- Measure plate at OD 570 nm for colorimetric assay or at Ex/Em= 535/587 nm for fluorometric assay.

17. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

18. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Avoid EDTA and other chelators when preparing plasma or serum samples.
- Sodium Fluoride (NaF)

19. FAQs

Q. Why is EDTA-plasma not ideal for this assay?

A. EDTA is not ideal for measurement of HDL/LDL in samples. EDTA plasma has potential disadvantages that have discouraged its use: inadequate mixing with EDTA can result in microclots; also, EDTA osmotically draws water from red cells, diluting the plasma constituents. We recommend using heparin when collecting sample, although other anti-coagulants such as citrate can also be used.

If you have used EDTA to collect the blood, we recommend precipitating the serum sample with the 2X Precipitation Buffer provided and then proceed with the protocol as described in the sample preparation and assay procedure sections.

Q. Can the separated HDL/LDL/VLDL be used for LC-MS or HPLC?

A. We have not tested the components in an HPLC system. This is optimized as a cell based assay in a plate format. Components in the precipitation buffer can potentially interfere in HPLC or LC-MS applications.

Q. Why is there higher free LDL than total LDL in some samples?

A. There are couple of possibilities for that phenomenon:

1. After treatment with the esterase, the free cholesterol in these samples is found associated to membrane/lipid, thus making it difficult to detect.

2. The cholesterol esters can be associated with serum lipids in the samples and therefore be inaccessible for the esterase to convert them to free cholesterol. Adding a small volume of detergent to solubilize the lipids might help. This could increase the reading for the total cholesterol.

Add NP-40 at 0.05% concentration in the assay buffer and then mix with the sample before adding to each well; the idea being to keep the lipids solubilized and not in rafts/other lipid structures trapping the cholesterol in them.

Additionally, if there is any sodium fluoride (NaF) in the sample (NaF being frequently used as a phosphatase inhibitor), it can inhibit the cholesterol esterase which could lead to these results.

20. Notes

Technical Support

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