ab102515

Cholesterol/Cholesteryl Ester Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of free cholesterol, cholesteryl esters, or both in various samples.

View kit datasheet: www.abcam.com/ab102515
(use www.abcam.cn/ab102515 for China, or www.abcam.co.jp/ab102515 for Japan)

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

Version: 6b, Last Updated: 2 July 2025

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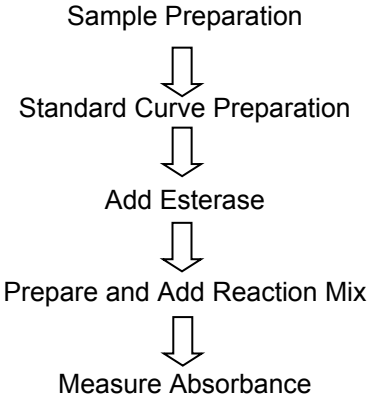
1. Overview

Cholesterol is an essential molecule in all animal life. It has been involved in both normal development and diseases. Abcam's Cholesterol/Cholesteryl Ester Detection Kit provides a simple method for sensitive quantification of free cholesterol, cholesteryl esters, or both using a colorimetric method. Most of the cholesterol in blood is in the form of cholesteryl ester. These esters can be hydrolyzed to free cholesterol under the appropriate conditions. In the assay, free cholesterol is oxidized by cholesterol dehydrogenase to generate NADH which reacts with a sensitive probe resulting in strong absorbance at 450 nm.

The assay can detect free or total cholesterol depending upon whether esterase is utilized to hydrolyze cholesterol esters present. Cholesteryl ester can be determined by subtracting the value of free cholesterol from the total cholesterol (cholesterol plus cholesteryl esters). The probe in this kit is more stable, sensitive and specific. The assay can tolerate interferences from various samples significantly.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer 2	25 mL
Developer Solution III	1 Vial
Cholesterol Enzyme Mix	1 Vial
Cholesterol Esterase	1 Vial
Cholesterol Standard	100 µL

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Cholesterol Assay Buffer, and Developer Solution III as Substrate Mix (Lyophilized), and Cholesterol Enzyme Mix was previously labelled as Enzyme Mix (Lyophilized), and Cholesterol Esterase was previously labelled as Esterase (Lyophilized), and Cholesterol Standard was previously labelled as Cholesterol Standard (2 µg/µl). The composition has not changed.

* Store kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Keep enzymes and cholesterol standard on ice while using. Read the entire protocol before performing the assay.

DEVELOPER MIX III: Reconstitute with 220 µl of Assay Buffer 2 and mix thoroughly. The solution is stable for 2 months at 4°C.

Cholesterol Enzyme Mix: Dissolve in 220 µl Assay Buffer 2 before use. Aliquot and store at -20°C. Use within two months.

CHOLESTEROL ESTERASE: Dissolve in 220 µl Assay Buffer 2 before use. Aliquot and store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a) Serum should be diluted 10X with Assay Buffer 2, and then use 2-20 µl for each testing.

For cells or tissue samples, extract 10^6 cells or 10 mg tissue with 200 µl $CHCl_3$: IPA:NP-40 (7:11:0.1) in a microhomogenizer. Centrifuge the extract for 5 min at 15,000 x g.

b) Transfer the liquid phase to a new tube and air dry at 50°C then place samples under vacuum for 30 min to remove any remaining solvent.

c) Dissolve dried lipids with 200 µl of Assay Buffer 2 by sonicating or vortexing until homogeneous (OK if the solution becomes cloudy). The extraction procedure can be scaled up if larger amounts of sample are desired. Use 1- 50 µl of extract per assay.

d) Adjust volume to 50 µl/well with Assay Buffer 2.

For unknown samples, we suggest testing different amounts of sample to ensure that readings are within the limits of the standard curve.

2. Standard Curve Preparation:

Dilute the Cholesterol Standard to 0.25 µg/µl by adding 20 µl of the Cholesterol Standard to 140 µl of Assay Buffer 2, mix well. Add 0, 4, 8, 12, 16, 20 µl into a series of wells.

Adjust volume to 50 µl/well with Assay Buffer 2 to generate 0, 1, 2, 3, 4, 5 µg/well of the Cholesterol Standard.

3. Add 2 µl Cholesterol Esterase to each standard and samples for which the total cholesterol value is desired. (See Notes a and b in step 4). Incubate 30 min at 37°C.

4. Reaction Mix Preparation: Mix enough reagent for the number of assays performed: For each well, prepare a total 48 µl Reaction Mix containing:

Assay Buffer 2	44 µl
Developer Solution III	2 µl
Cholesterol Enzyme Mix	2 µl

Mix well. Add 48 µl of the Reaction Mix to each well containing standard or samples.

Notes:

a) Cholesterol Esterase hydrolyzes cholesteryl ester to cholesterol. If you want to detect free cholesterol only, omit the Cholesterol Esterase in the reaction (step 3) and adjust the volume of reaction mix to 50 µl by adding 46 µl Assay Buffer 2. In the presence of Cholesterol Esterase, the assay detects both free cholesterol and cholesteryl esters. If you want to determine Cholesteryl Ester only, subtract the value of free cholesterol from the value of total cholesterol (Cholesterol and Cholesteryl Ester).

b) The Cholesterol Standard contains a mixture of free cholesterol and cholesterol esters in a similar ration of serum. Cholesterol Esterase must be added to the standard reaction to convert all cholesterol in the standard.

5. Incubate the reaction for 30 min at 37°C, protect from light.

6. Measure absorbance at 450 nm in a microplate reader.

5. Data Analysis

Subtract the zero standard background reading from all readings.

Plot the standard curve. Apply sample readings to the standard curve.

Cholesterol concentration in samples can then be calculated:

Concentration = A / V X D (µg/µl)

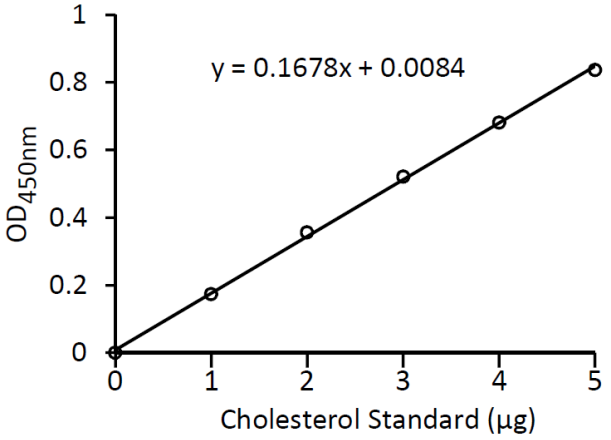
Where:

A = amount of cholesterol determined from Standard Curve (in µg).

V = volume of sample added into the reaction well (in µl).

D = sample dilution factor.

Cholesterol molecular weight: 386.65. 1 µg/µl = 100 mg/dL.



Cholesterol/Cholesteryl Ester was quantified according to the kit instructions. Background from the zero standard reading (without cholesterol) has been subtracted from all readings.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)