Version 3b Last updated 21 June 2024

ab242307
Lipid Assay Kit (neutral lipids)

For the quantitation of neutral lipids in plasma, serum and tissue or cultured cell samples.

View kit datasheet: www.abcam.com/ab242307

(use www.abcam.cn/ab242307 for China, or www.abcam.co.jp/ab242307 for Japan)

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

Table of Contents

1. Overview

Lipids are a diverse group of molecules that include monoglycerides, diglycerides, triglycerides, fats, sterols, and others. Not only do lipids define and preserve cellular membrane integrity, but they are also involved in cellular processes such as membrane trafficking, signal transduction, apoptosis, and energy storage. Perturbation in the metabolism of lipids has been linked to many diseases such as cancer, diabetes, Alzheimer's disease, and coronary heart disease. In order to study lipids, they must often be extracted first from tissues or cellular cultures and then quantified. Some methods for lipid quantification have been described that are quite sensitive, however they require expensive equipment such as an HPLC machine, light scattering detection technology, or a Iatroscan TLC-FID analyzer.

ab242307 specifically measures the neutral lipid content of samples using a lipid binding molecule that fluoresces brightly only when bound to lipids, resulting in a simple fluorescence readout. First, a crude or purified lipid source is applied to a 96-well plate. Then organic solvents are removed, followed by the addition of the fluorescent reagent. Lipid binding to the fluorescent reagent results in an increase in fluorescence that is easily detected on a microplate reader. This kit has a detection sensitivity of 3.9 mg/dL and is at least 10 times more sensitive than the Vanillin method. Each kit provides sufficient reagents to perform 100 assays including standards and unknown samples.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.

Add 40 µL of Lipid Standard or sample to wells of microtitre plate.

Incubate the plate at 55°C for 20-30 minutes

Incubate the plate at 4°C for 2-3 minutes.

Add 40 µL of isopropanol. Mix the well.

Add 200 µL of 1X Fluorometric Reagent.

Incubate for 5-15 minutes at room temperature protected from light.

Read fluorescence at Ex/Em = 490 nm / 585 nm

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at 4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Fluorometric Reagent (100X)	200 µL	4°C
Lipid Standard	100 µL	4°C

5. Materials Required, Not Supplied

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

- Methanol
- Chloroform
- Isopropanol
- Standard 96-well fluorescence black microtiter plate
- Fluorescence microplate reader capable of reading Ex/Em = 490/585 nm

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 1X Fluorometric Reagent

6.1.1 Thaw the Fluorometric Reagent (100X) stock at 37°C for 5 minutes.

6.1.2 Dilute 100-fold with water.

6.2 Methanol/Chloroform Mixture (only for testing organic phase samples)

6.2.1 Mix 2 volumes of methanol with 1 volume of chloroform in a glass bottle.

6.2.2 Store at room temperature.

ΔNote: Methanol/Chloroform Mixture is toxic. Avoid skin contact or inhalation and use a fume hood for preparation.

7. Standard Preparation

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

7.1 Preparation of the Lipid Standard Curve

7.1.1 Prepare a dilution series of the Lipid Standard in the concentration range of 0 – 500 mg/dL by diluting the Lipid Standard stock solution in either water or chloroform/methanol.

ΔNote: If testing aqueous phase samples (plasma, serum or purified lipoproteins), prepare standards in distilled water.

ΔNote: If testing organic phase samples (lipid extracts or purified lipids), prepare standards in Methanol/Chloroform Mixture (see Reagent Preparation).

Standard#	20 g/dL Lipid Standard (µL)	Water or Chloroform/Methanol (µL)	Final Lipid Standard (mg/dL)
1	10	390	500
2	200 of standard #1	200	250
3	200 of standard #2	200	125
4	200 of standard #3	200	62.5
5	200 of standard #4	200	31.3
6	200 of standard #5	200	15.6
7	200 of standard #6	200	7.8
8	200 of standard #7	200	3.9
9	0	200	0

8. 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 for the most reproducible assay.

8.1 Plasma:

8.1.1 Collect blood with an anticoagulant such as citrate, EDTA, heparin or oxalate and mix by inversion.

8.1.2 Centrifuge the blood at 1000 x *g* at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer.

8.1.3 Sample should be tested immediately or frozen at -80°C for storage. Dilute as necessary in water.

8.2 Serum:

8.2.1 Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes.

8.2.2 Centrifuge at 2500 x *g* for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer.

8.2.3 Samples should be tested immediately or frozen at -80°C for storage. Dilute as necessary in water.

8.3 Cultured Cells or Tissues:

8.3.1 Prepare lipids using the Folch method.

8.3.2 Dilute sample, as required, in chloroform/methanol.

ΔNote: Samples containing chloroform should be diluted in methanol to less than 33% before adding, due to the incompatibility of higher chloroform concentrations with polystyrene microtiter wells.

9. Assay Procedure

– We recommend that you assay all standards, controls and samples in duplicate.

9.1 For aqueous phase samples (plasma, serum, or purified lipoproteins):

9.1.1 Add 40 µL of the Lipid Standards and samples to the 96-well microtiter plate.

9.1.2 Add 200 µL of 1X Fluorometric Reagent and pipette up and down 5 times to mix.

9.1.3 Incubate at room temperature for 5-15 minutes protected from light.

9.1.4 Read the plate at 490 nm excitation and 585 nm emission with a fluorescent plate reader.

9.2 For organic phase samples (lipid extracts or purified lipids):

9.2.1 Add 40 µL of the Lipid Standards and samples to the 96-well microtiter plate.

9.2.2 Incubate the plate at 55°C for 20-30 minutes to fully evaporate the organic solvent.

9.2.3 Incubate the plate at 4°C for 2-3 minutes.

9.2.4 Add 40 µL of isopropanol. Mix the well by pipetting up and down 10 times.

9.2.5 Add 200 µL of 1X Fluorometric Reagent and pipette up and down 5 times to mix.

9.2.6 Incubate at room temperature for 5-15 minutes protected from light.

9.2.7 Read the plate at 490 nm excitation and 585 nm emission with a fluorescent plate reader.

10. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #9) from all standards and samples.
3. Plot the corrected values for each standard as a function of the final concentration of Lipid Standard.
4. Calculate the concentration of lipids within samples by comparing the sample fluorescence to the standard curve.

11. Typical Data

One should use the data below for reference only. This data should not be used to interpret actual results.

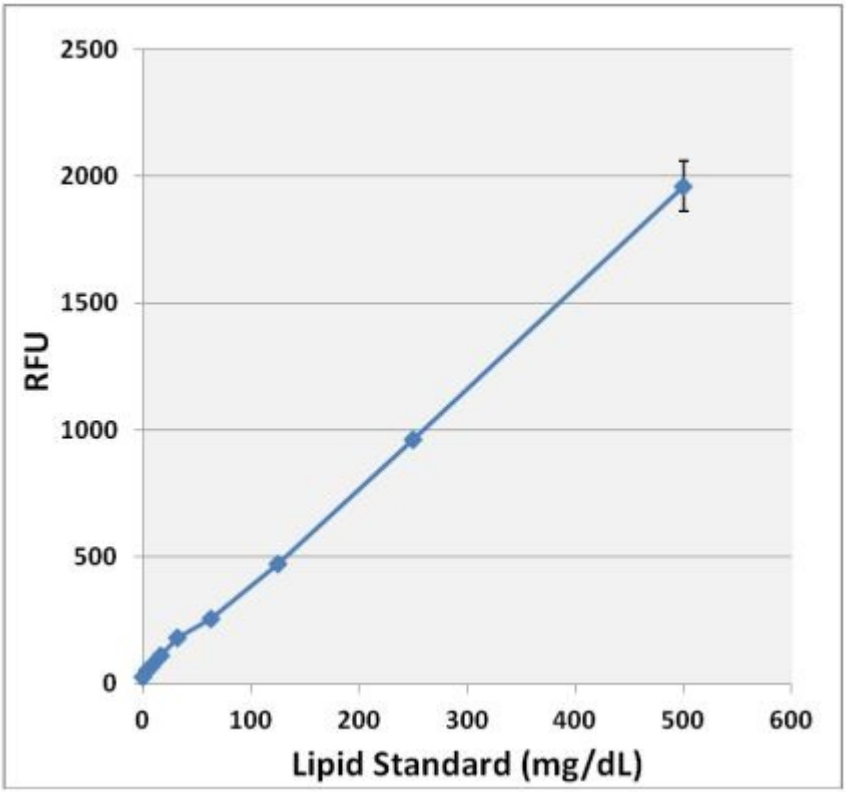

Figure 1. Lipid Standard curve in methanol/chloroform.

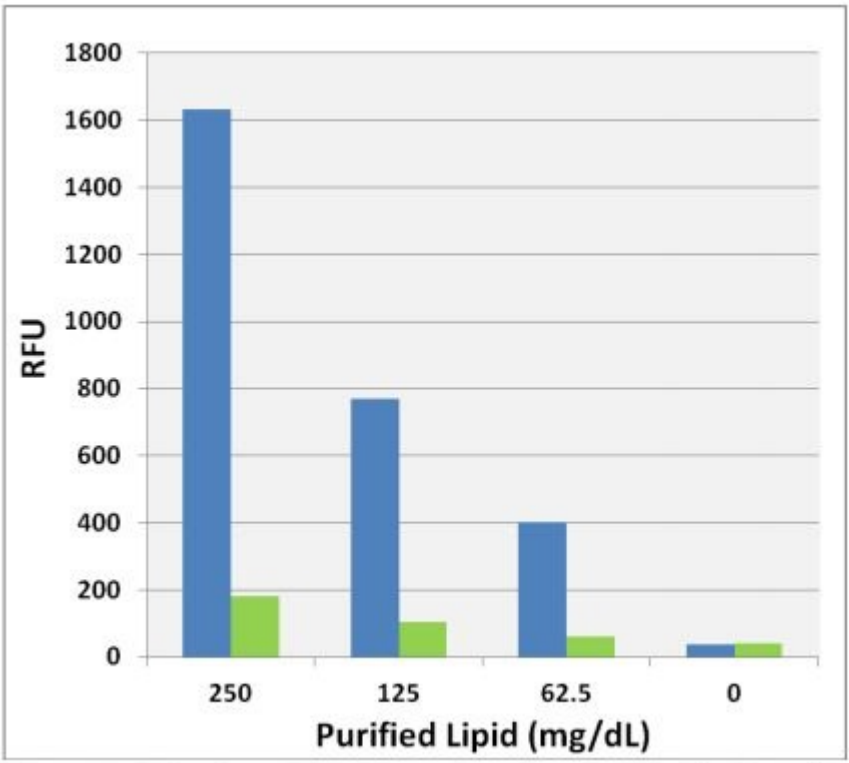

Figure 2. Detection of triolein or lysophosphatidylcholine. Purified triolein (blue bars) or lysophosphatidylcholine (green bars) were diluted in Methanol/Chloroform.

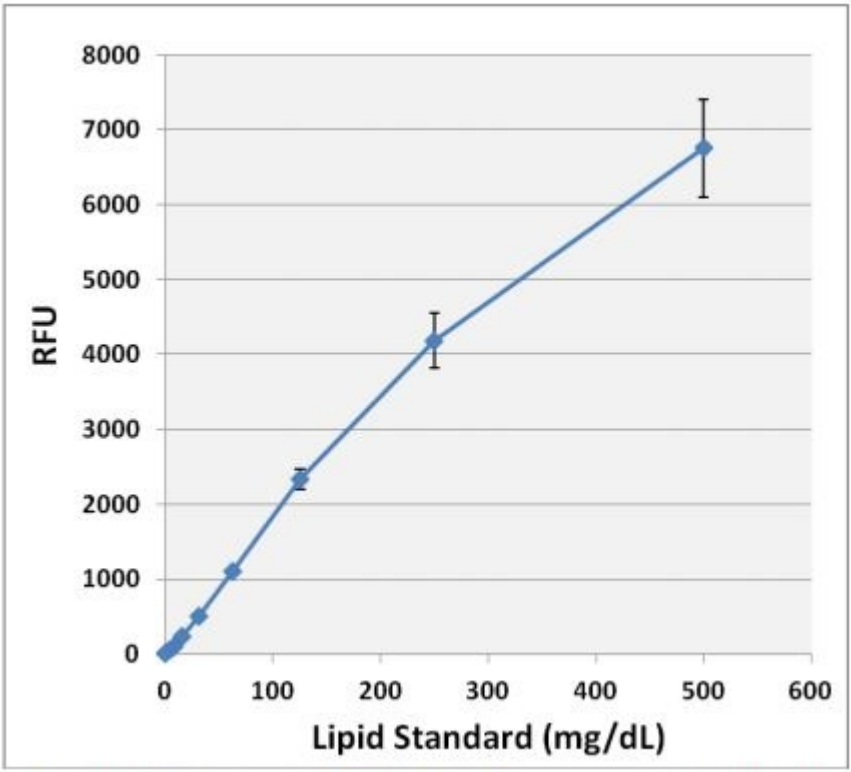

Figure 3. Lipid Standard curve in water.

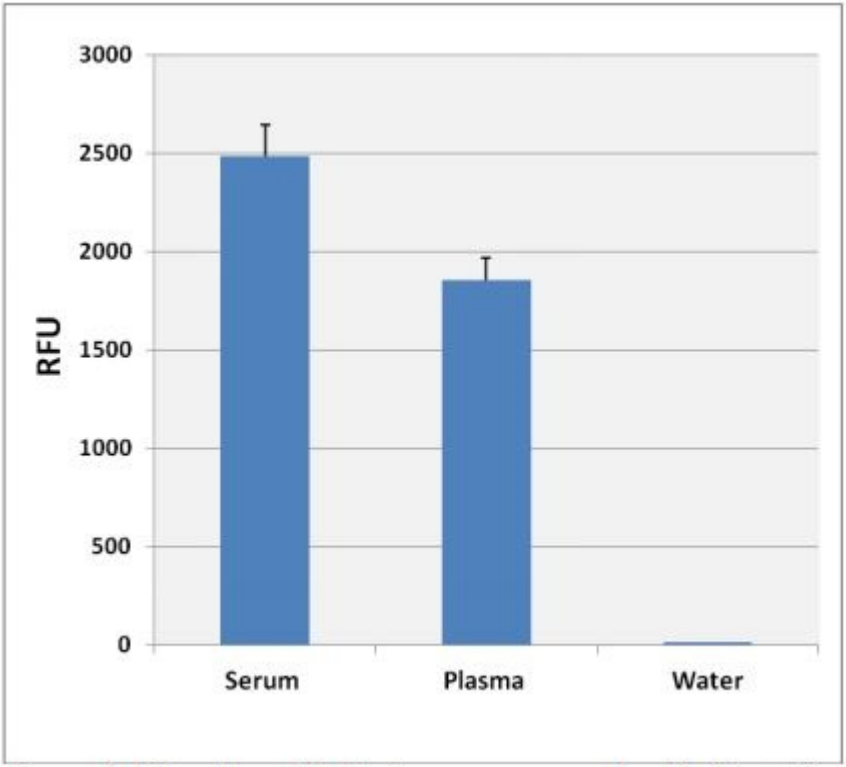

Figure 4. Detection of lipids in aqueous samples. Undiluted human serum, human plasma, and water were analyzed.

12. Notes

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)