Version 2c Last updated 23 July 2021

ab242305 Lipid Assay Kit (unsaturated fatty acids)

View Lipid Assay Kit (unsaturated fatty acids)datasheet: www.abcam.com/ab242305

For the measurement of lipid content (unsaturated fatty acids only) from extracted lipid sources.

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

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1. Overview

The Lipid Assay Kit (unsaturated fatty acids) (ab242305) measures the lipid content (unsaturated fatty acids only) of samples using the sulfo-phospho-vanillin method resulting in a simple colorimetric readout amenable to multi-well plate detection.

First, a crude or purified lipid source is applied to a 96 well plate. Then concentrated sulfuric acid is added and the samples are heated to solubilize and prime the total lipid sample, followed by the addition of vanillin in an acid solution. The lipids react with vanillin in the presence of the acids to form a colorimetric product that is easily detected on a microplate reader.

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 15 µL of standards or unkown samples into a 96-well plate. Incuate samples at 90°C for 30 minutes.

Transfer samples to 4°C for 5 minutes. Add 150 µL of 18M sulfuric acid.

Incubate samples at 90°C for 10 minutes then at 4°C for 5 minutes.

Transfer 100 µL of each standard and sample into a clean 96-well plate and read plate at 540 nm to determine the background.

Add 100 µL of Vanillian Reagent and mix. Incubate at 37°C for 15 minutes. Read samples at OD 540 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 -20°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
Purified lipid Standard	100 µL	-20°C
Vanillin Reagent	10 mL	-20°C

5. Materials Required, Not Supplied

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

- Concentrated Sulfuric Acid (18M)
- DMSO or other organic solvent
- Glass tubes, 15 mL conical tubes, microcentrifuge tubes, or 96 well plates
- 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 1000 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir

6. Reagent Preparation

– All regents in this kit are ready to use as supplied.

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 Thaw the Purified Lipid Standard at room temperature and prepare a dilution series of Purified Lipid Standards in the concentration range of 0 to 2500 mg/dL in dimethyl sulfoxide (DMSO) or desired solvent, see table below:

Standard #	100 g/dL Purified Lipid Standard (µL)	DMSO or other organic solvent (µL)	Standard (mg/dL)
1	10	390	2,500
2	200 of tube #1	200	1,250
3	200 of tube #2	200	625
4	200 of tube #3	200	313
5	200 of tube #4	200	156
6	200 of tube #5	200	78
7	200 of tube #6	200	39
8	0	100	0

8. Sample Preparation

- The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

8.1 Plasma:

- Collect blood with an anticoagulant such as citrate, EDTA, heparin or oxalate and mix by inversion.
- Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer.
- Sample should be tested immediately or frozen at -80°C for storage.

8.2 Serum:

- Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes.
- Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer.
- Samples should be tested immediately or frozen at -80°C for storage.

8.3 Cultured Cells or Tissue Samples:

- Prepare lipids using The Lipid Extraction Kit (Chloroform Free) (ab211044).

9. Assay Procedure

ΔNote: Sulfuric acid is highly corrosive and can damage certain types of plastics. Avoid using plastics that are sensitive to sulfuric acid, and test plastics prior to attempting this assay by adding 100 µL of sulfuric acid and heating to 90°C for 10 minutes. Sulfuric acid should be handled with care. Gloves, a lab coat, and protective eyewear should be worn during handling. Sulfuric acid should be stored in glassware only and be pipetted in a fume hood.

9.1 Add 15 µL of samples or standards into microcentrifuge tubes or a 96-well plate.

ΔNote: For samples in DMSO, including the standards, skip steps 9.2 and 9.3 and proceed to step 9.4.

9.2 Incubate samples and standards uncovered at 90°C for 30 minutes to completely evaporate organic solvents.

ΔNote: This step is optional for aqueous, non-organic based samples, which will not evaporate during heating.

9.3 Transfer samples to 4°C for 5 minutes.

9.4 Add 150 µL of 18M sulfuric acid.

9.5 Incubate samples at 90°C for 10 minutes.

9.6 Transfer samples to 4°C for 5 minutes.

9.7 Transfer 100 µL of each standard and unknown sample into a clean 96-well plate.

9.8 Read samples at OD 540 nm to determine background.

9.9 Add 100 µL of Vanillin Reagent and mix carefully.

ΔNote: The Vanillin Reagent tends to precipitate and may require incubation at 37°C for 15-30 minutes to go into solution.

9.10 Incubate samples at 37°C for 15 minutes.

9.11 Read samples at OD 540 nm to determine signal.

9.12 Subtract background from signal.

10. Typical Data

The following figure demonstrates typical blot results for the CML-BSA Immunoblot Control. One should use the data below for reference only. This data should not be used to interpret actual results.

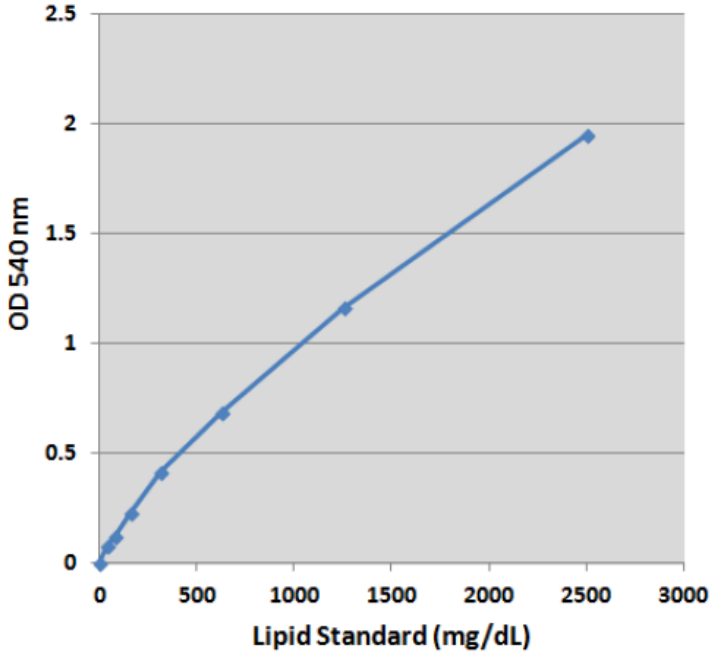

Figure 1. Lipid Standard Curve.

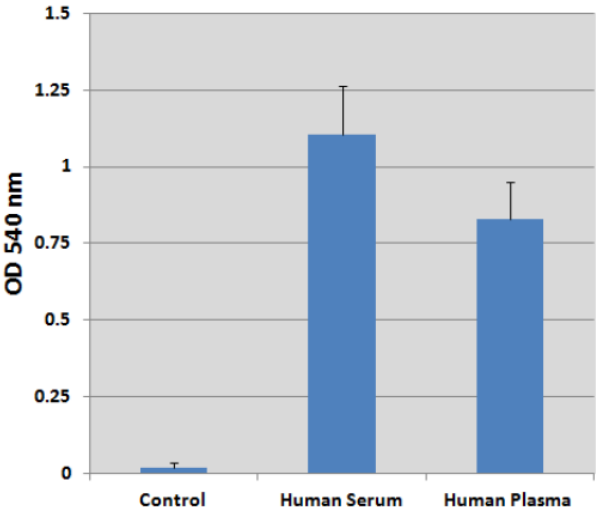

Figure 2. Detection of lipids from Human Serum or Human Plasma. Fifteen microliters of undiluted Human Serum, Human Plasma, or Negative Control buffer were analyzed using the Lipid Quantification Kit (Colorimetric).

11. 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)