Product Information

Free Fatty Acid Quantification Colorimetric/Fluorometric Kit

I. Kit Contents:

Components	K2121-100 100 assays	Cap Color	Part Number
Fatty Acid Assay Buffer	25 ml	WM	K2121-C-1
Fatty Acid Probe (in DMSO)	200 µl	Red	K2121-C-2
ACS Reagent	1 vial	Blue	K2121-C-3
Enzyme Mix	1 vial	Green	K2121-C-4
Enhancer	200 µl	Purple	K2121-C-5
Palmitic Acid Standard (1nmol/µl)	300 µl	Yellow	N2456

II. Introduction:

Fatty Acid plays an important role in metabolism and many disease developments and is usually derived from phospholipid or triglyceride. Fatty Acids are precursors to many bioactive compounds such as leucotrienes and prostaglandins. Fatty Acids are involved in autism, inflammation response and immune system.

The Free Fatty Acid Quantification Colorimetric/Fluorometric Kit provides a sensitive, fast and convenient way for detection of long-chain free fatty acids in various biological samples such as plasma, serum, other body fluids, growth media and food based on colorimetric and fluorometric method. In the assay, free fatty acids are converted to their CoA derivatives, which are then oxidized with the production of fluorescence (Ex/Em = 535/587 nm) and color (λmax = 570 nm). C-8 (octanoate) and longer fatty acids can be easily detected with detection limit 2 µM in variety samples.

III. Storage and Handling:

Store kit at –20°C, protect from light. Allow Fatty Acid Assay Buffer warm to room temperature before use.

IV. Reagent Preparation:

Probe: Ready to use as supplied. Warm to room temperature prior to use to melt frozen DMSO. Store at –20°C, protect from light and moisture. Use within two months.

Enzymes: Dissolve ACS (Acyl-CoA Synthetase) Reagent and Enzyme Mix individually with 220 µl Assay Buffer each by pipetting up and down. Store at –20°C. Use within two months.

Palmitic Acid Standard: Frozen storage may cause the Palmitic Acid Standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed, place in a hot water bath (~80-100°C) for 1 min. or until the standard looks cloudy, vortex for 30 sec., the standard should become clear. Repeat the heat and vortex one more time. The Palmitic Acid Standard is now completely in solution, and ready to use.

V. Free Fatty Acid Assay Protocol:

The following protocol describes assays in 100 µl per microplate well.

1. Standard Curve Preparation:

For the colorimetric assay, add 0, 2, 4, 6, 8, 10 µl Palmitic Acid Standard into 96-well plate individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Fatty Acid Standard.

For the fluorometric assay, dilute the Palmitic Acid Standard to 0.1 nmol/µl by adding 10 µl of the Standard to 90 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Fatty Acid Standard.

2. Sample Preparation: For testing liquid samples, different volume of samples can be directly added to each well in a 96-well plate, then bring up the volume to 50 µl/well with Assay Buffer. For unknown samples, we suggest using different doses to ensure the readings are within the standard curve range.

For testing cell or tissue samples, 10^6 cells or 10 mg tissue samples can be extracted by homogenization with 200 µl of chloroform-Triton X-100 (1% Triton X-100 in pure chloroform) in a microhomogenizer. Then spin the extract 5-10 minutes at top speed in a microcentrifuge. Collect organic phase (lower phase), air dry at 50°C to remove chloroform. Vacuum dry 30 min to remove trace chloroform. Dissolve the dried lipids (in Triton X-100) in 200 µl of Fatty Acid Assay Buffer by vortexing extensively for 5 mins (Note: The solution may be slightly turbid or opalescent, but this does not affect the assay). The extraction procedure can be proportionally scaled up if larger amount of sample is desired. Use 1- 50 µl of the extracted sample per assay.

3. Acyl-CoA Synthesis: Add 2 µl ACS Reagent into all the standard and sample wells. Mix well; incubate the reaction at 37°C for 30 min.

4. Reaction Mix Preparation: Mix enough reagents for the number of assays and standard performed: For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer	44 µl
Fatty Acid Probe	2 µl
Enzyme Mix	2 µl
Enhancer	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the Standard or test samples. Incubate the reaction for 30 min at 37°C, protect from light.

6. Measure O.D. 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a micro-plate reader.

7. Calculation: Subtract background value (the 0 Control) from all standard and sample readings. Plot standard curve nmol/well vs. OD570nm or fluorescence readings. Then apply the sample readings to the standard curve to obtain Fatty Acid amount in the sample wells.

Fatty Acid Concentration = Fa/Sv (nmol/µl or mM).

Fa is the Fatty Acid amount (nmol) in the well obtained from standard curve.

Sv is the sample volume (µl) added to the sample well.

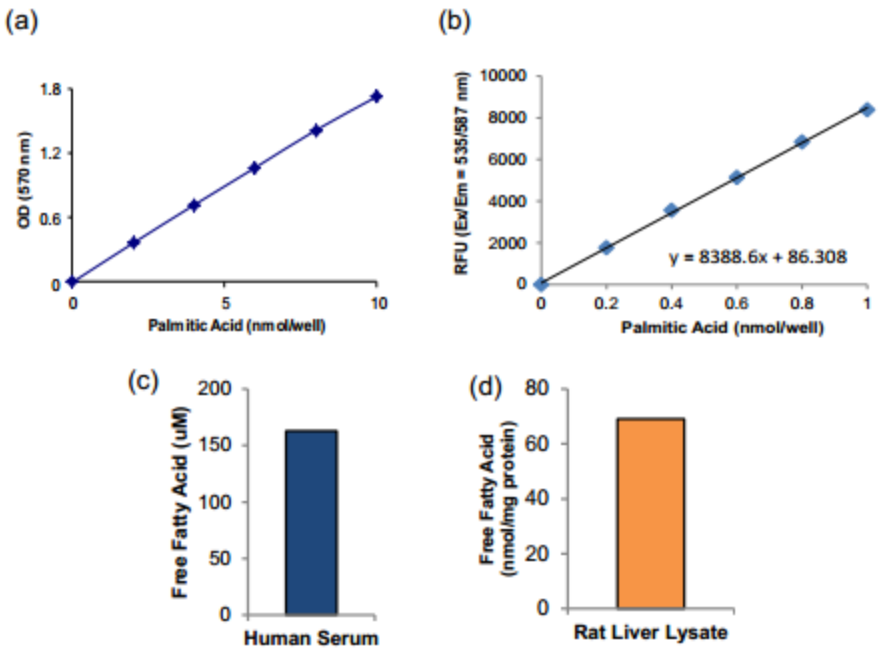

Figure: Palmitic Acid Standard Curve: Colorimetric (a), Fluorometric (b). Quantitation of free fatty acid in pooled human serum (10 µl) (c) and rat liver lysate (50 µg) (d). Assays were performed following the kit protocol.

Frequently Asked Questions:

1. Which anticoagulants interfere with this assay and which can be used?

Blood needs to be collected using an anticoagulant such as EDTA, sodium citrate, sodium fluoride, or ammonium oxalate. Heparinized plasma is not the best choice as high amounts of heparin could interfere with the assay.

2. What is the concentration of fatty acid in human serum?

Normal Human Serum has 80 - 250 µg/ml Fatty acid.

3. Why is there a large background even after using the correct Excitation/Emission settings?

It seems that the problem is the fluorescence reading overlap between excitation an emission readings for the particular instrument. If a fluorometer has window of sensitivity +/-40 nm, the excitationa nd emission overlap and then oit is needed to resort to a longer wavelength for emission which is above 600 nm (Some times up to 620 nm).

4. Will the phenol red in the media affect the assay readout?

Very low amounts of media are used for each sample. This will generate a very low background at the best. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

5. What is the length of fatty acid chains that can be quantified using this kit? A palmitic acid std is used, but are oleic and stearic acid chains able to be detected as well?

C-8 (octanoate) and longer fatty acids can be quantified. There is no difference upon measurement of chains C-16 and above.

6. What is the detection limit for this kit ?

The detection limit is ~2 μM fatty acid.

7. How long should samples be air-dried ?

Typically up to 1 hour is enough to air- dry at 50℃ to remove chloroform. The goal to remove as much chloroform at this step as possible.

8. Can protein content be used as an internal control for this assay?

Since the tissues/cells are homogenized in chloroform/Triton a protein assay is not recommended.

9. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

10. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com