Free Fatty Acid Quantification Colorimetric/Fluorometric Kit

(Catalog #K612-100; 100 assays; Store at -20°C)

I. Introduction:

Fatty Acids play very important roles in normal metabolism and many disease developments. They are precursors to a number of bioactive classes of compounds such as prostaglandins, leucotrienes and others, and have been implicated in diverse functions such as autism, immune system and inflammation response. BioVision's Free Fatty Acid Quantification Kit provides a convenient, sensitive enzyme-based method for detecting the long-chain free fatty acids in various biological samples, such as serum, plasma and other body fluids, food, growth media, etc. In the assay, Fatty Acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of color or fluorescence. C-8 (octanoate) and longer fatty acids can then be easily quantified by either colorimetric (spectrophotometry at λ = 570 nm) or fluorometric (at Ex/Em = 535/587 nm) methods with detection limit 2 µM free fatty acid in variety samples.

II. Kit Contents:

Components	100 Assays	Cap Cod	Part Number
Fatty Acid Assay Buffer	25 ml	WM	K612-100-1
Fatty Acid Probe (in DMSO)	200 µl	Red	K612-100-2A
ACS Reagent	1 vial	Blue	K612-100-4
Enzyme Mix	1 vial	Green	K612-100-5
Enhancer	200 µl	Purple	K612-100-6
Palmitic Acid Standard (1nmol/µl)	300 µl	Yellow	K612-100-7

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:

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

    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. OD_{570nm} 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.

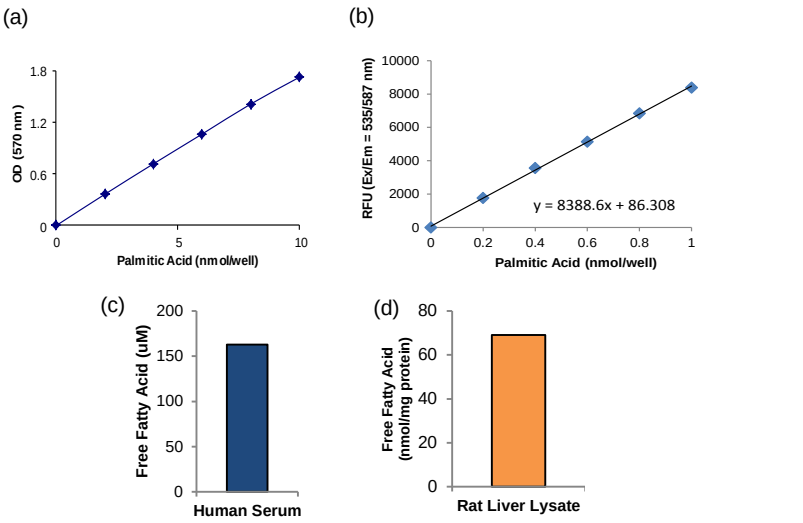

Figure: Palmitic Acid Standard Curve: Colorimetric (a), Fluorometric (b). Quantitation of free fatty acid in pooled human serum (10 µl) (c) and rat liver lysate (50 µg) (d). Assays were performed following the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

BioVision Incorporated
155 S. Milpitas Boulevard, Milpitas, CA 95035 USA

Tel: 408-493-1800 | Fax: 408-493-1801
www.biovision.com | tech@biovision.com