MA-0111

Free Fatty Acid Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Fatty Acids are hydrocarbon chains terminating in a carboxylic acid. They are important as a source of energy, as structural components and as precursors to a variety of bioactive chemicals collectively referred to as oxylipins with a diverse set of biological actions, including pro- and anti-inflammatory, vasoactive, autocrine and paracrine functions. AkrivisBio's Free Fatty Acid Assay provides a simple, sensitive method for measuring free fatty acids in a variety of biological samples. The assay is based upon the conversion of fatty acids to CoA derivatives which are oxidized with concomitant generation of a stoichiometric amount of hydrogen peroxide which is used to oxidize ADHP giving rise to color and fluorescence proportional to the fatty acid content. An enhancer is added to the reaction to increase color and fluorescence development.

Assay Principle:

1 - Fatty acid is converted to AcylCoA.
2 – The AcylCoA is oxidized with the formation of hydrogen peroxide
3 - Hydrogen peroxide is utilized by peroxidase to oxidize ADHP to resorufin resulting in intense color and fluorescence

Assay Components:

Assay Buffer	25 ml	WM	MA-0111A
ADHP Solution	200 µl	Red	MA-0111B
Acyl CoA Synthase	lyophilized	Blue	MA-0111C
Acyl CoA Oxidase/HRP	lyophilized	Green	MA-0111D
Enhancer	200 µl	Purple	MA-0111E
Palmitic Acid Standard (1 mM)	300 µl	Yellow	MA-0111F

User Supplied Materials:

Lipid extraction materials (See **AkrivisBio PI-0101** Lipid Extraction Kit)
Polytron or homogenizer
Filters

Storage and Handling:

Store kit at -20°C. Allow all components to come to room temperature before using.

ADHP Solution: DMSO freezes just below room temperature. It must be brought to room temperature before use. Store at -20°C.

Acyl-CoA Synthetase; Acyl CoA Oxidase/HRP: Reconstitute lyophilized enzymes with 220 µl DI water and mix. Store at -20°C.

Palmitic Acid Standard: Freezing of the Palmitic Acid standard can result in separation into two phases. To prepare it for use, heat it to above 80°C in a hot water bath (~80-100°C) until the solution is above its cloud point where the solution becomes very turbid. While hot, vortex. As the solution cools it will once again become clear. Repeat the heating/cooling cycle one time and the Standard is ready to use.

Assay Protocol:

1. Standard Curve:
Absorbance based assay (0 – 10 nmol/well range): Transfer 0-5-10-15-20-25 µl of the Palmitic Acid Standard into a series of wells of a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer. This gives 0-2-4-6-8-10 nmoles per well of the Standard.
Fluorescence based assay (< 1 nmol/well): Dilute the palmitic acid standard 10X to 40 µM by adding 50 µl of the Standard to 450 µl of Assay Buffer, then transfer 0–5–10–15–20-25µl of the Standard to wells of a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 200 – 400 – 600 – 800 - 1000 pmoles per well of the Standard.

2. Sample Preparation:
Liquid samples can be added directly to a 96 well plate and all well volumes adjusted to 50 µl with Assay Buffer. Unknown samples must give a response within the limits of the standard curve being used. If an unknown sample is outside of this range, it must be diluted and rerun.
Cell (10^6) or tissue (10 mg) are extracted with a 5% isopropanol/5% Tergitol solution in a small homogenizer then filtered to remove insoluble and cell debris. The sample is ready to use. The solution may be turbid, but this does not affect the assay. Use 1- 20 µl of sample per assay.

3. Acyl-CoA Synthesis: Add 2 µl Acyl CoA Synthase into all standard and sample wells. Mix and incubate for 30 minutes at 37°C.

4. Oxidation and Color Development Reaction Mix: Prepare enough Reaction Mix for the number of wells to be run(samples and standards). For each well, prepare a total of 50 µl of Reaction Mix containing:

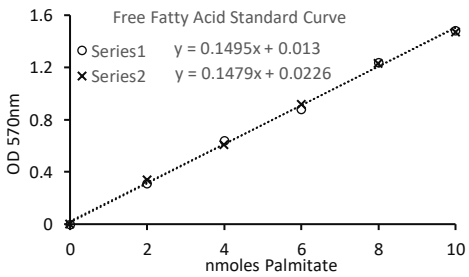
Assay Buffer	44 µl
Acyl CoA Oxidase/HRP	2 µl
ADHP Solution	2 µl
Enhancer	2 µl

Add 50 µl of the Reaction Mix to each well. Incubate the reaction for 30 min at 37°C, protected from light. It can be useful to observe the color or fluorescence development as it proceeds by measuring the reaction in kinetic mode in a plate reader.

5. Measure Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less than 30 minutes to reach endpoint.

6. Typical Results:

nmole	Standard Raw Values		Background Corrected Values	
Standard	OD			
0	0.0626	0.0676	0	0
2	0.3737	0.4056	0.3111	0.3380
4	0.7031	0.6797	0.6405	0.6121
6	0.9459	0.9861	0.8833	0.9185
8	1.3033	1.2999	1.2407	1.2323
10	1.5488	1.5410	1.4862	1.4730



48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com

7. **Calculation:** Subtract OD of the 0 nmole Standard from all readings. Plot the Standard curve. The slope defines the sensitivity of the system (OD/nmole FFA). Divide the background corrected sample readings by the slope to determine nanomoles of FFA content. To convert back to content in the original sample:
   Original FFA Content = nmoles determined from standard curve slope and unknown OD Corrected for dilution:
   1- nmoles FFA determined / volume of sample added to wells = nmoles/µl of FFA
   2- nmoles /µl of FFA X total volume of sample = total nmoles FFA
   3- total nmoles FFA/ number of cells or mg of tissue = nmoles FFA/cell of nmoles FFA/mg tissue

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.