EnzyFluo™ Fatty Acyl-CoA Assay Kit (EFCOA-100)

Quantitative Fluorimetric Fatty Acyl-CoA Determination

DESCRIPTION

*Fatty Acyl-CoA*s are a combination of Coenzyme A covalently bound to long chain fatty acids. Fatty acyl-CoAs serve as both a precursor for triglyceride and phospholipids synthesis, and a product of lipid catabolism. Catabolically, they facilitate the transport of fatty acids to the mitochondria for shuttling via the carnitine shuttle and subsequent β-oxidation of the fatty acid. Conversion of fatty acids to fatty acyl-CoAs is necessary for generation of ATP during periods of high energy demand. Elevated fatty acyl-CoA levels may indicate dysregulation of lipid metabolism due to insulin resistance, for example. Certain genetic disorders may also affect fatty acyl-CoA levels.

BioAssay Systems' fluorimetric fatty acyl-CoA assay works using a combination of enzymes that utilize fatty acyl-CoA as a substrate, which is coupled to form a fluorescent product. The resulting fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is linear to the fatty acyl-CoA concentration in the sample.

KEY FEATURES

Sensitive and accurate. Use as little as 10 µL samples. Linear detection range in 96-well plate: 0.3 to 100 µM fatty acyl-CoA. Internal standard method minimizes potential sample matrix interferences.

Fast and convenient. Room temperature assay with a 40 min incubation. No 37°C incubator is needed.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of fatty acyl-CoAs in tissue and cell lysates.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Dye Reagent:**	120 µL
Enzyme:	100 µL	**Standard:**	Dried
HRP:	100 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation: Reconstitute dried palmitoyl-CoA (PCoA) standard to 5 mM by adding 20 µL methanol. To ensure the standard is fully dissolved, vortex the tube so that the methanol comes into contact with all of the dried standard. Store reconstituted standard for up to one month at -20°C.

Sample and Standard Preparation:

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove all blood. Homogenize tissue (50 mg) in ~150 µL buffer containing 0.5% Triton-X 100 and 20 mM potassium phosphate (pH 7.4). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 0.5% Triton-X 100 and 20 mM potassium phosphate (pH 7.4). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor *n*.

Samples should be assayed the same day if possible or immediately frozen at -80°C and tested within one week.

Note: SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol, > 5 µM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.

1. Sample Preparation. This internal standard method needs three separate reactions: 1) Sample plus Standard ("IS"), 2) Sample alone ("S") and 3) Sample Blank ("SB").

   Prepare 125 µM PCoA standard as needed by mixing the 5 mM Standard and dH_2O. For the "IS" wells, add 2 µL of 125 µM Standard to 10 µL of sample, mix well, and transfer 10 µL to the well. For "S" and "SB" wells, add 4 µL dH_2O. to 20 µL of sample, mix well, and transfer 10 µL to the "S" and "SB" wells, respectively.

2. Prepare Working Reagent (WR). For "IS" and "S" wells, prepare sufficient WR by mixing, for each well, 92 µL Assay Buffer, 1 µL Enzyme, 1 µL HRP, and 1 µL Dye Reagent. Prepare sufficient Blank Working Reagent (BWR) for the "SB" wells by mixing, for each well, 93 µL Assay Buffer, 1 µL HRP, and 1 µL Dye Reagent (no Enzyme).

   Add 90 µL WR and 90 µL BWR to the appropriate wells and tap the plate briefly to mix.

3. Read fluorescence ($\lambda_{ex/em}$ = 530/585 nm) immediately (0 min) and at 40 min, or record kinetics for 40 min.

CALCULATION

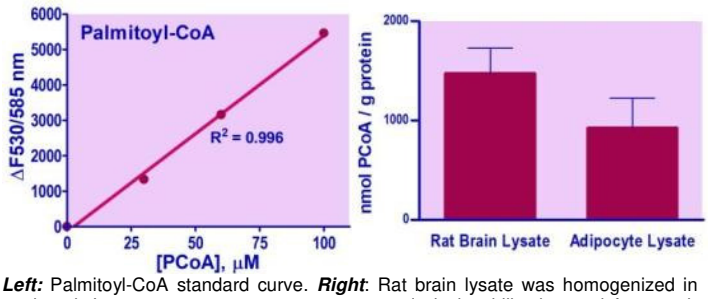
The sample's Fatty Acyl-CoA concentration is calculated as follows:

$$[\text{Fatty Acyl-CoA}] = \frac{\Delta F_S - \Delta F_{SB}}{\Delta F_{IS} - \Delta F_S} \times n \times 20.83 \ (\mu M)$$

Where ΔF_S, ΔF_{SB}, and ΔF_{IS} are the ΔF (40min - 0min) values of the Sample, Sample Blank and Sample plus Internal Standard, respectively. 20.83 is the spiked internal standard concentration. *n* is the sample dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black flat-bottom, uncoated 96-well plates (e.g. VWR cat# 82050-676), centrifuge tubes and plate reader. Methanol for dried standard reconstitution.



Left: Palmitoyl-CoA standard curve. ***Right***: Rat brain lysate was homogenized in methanol. Lysate supernatant was concentrated via lyophilization and fatty acyl-CoA levels were 1,472 ± 254 nmoles/g protein. Human adipocytes lysed in 5% Triton X-100 and 5% isopropanol, and fatty acyl-CoA levels were 924 ± 298 nmoles/g protein.

LITERATURE

1. Liu, X. et al. (2015). High-Resolution Metabolomics with Acyl-CoA Profiling Reveals Widespread Remodeling in Response to Diet. *Molecular & cellular proteomics*, *14*(6), 1489-1500.
2. Hesse, J. et al. (2018). The diagnostic challenge in very-long chain acyl-CoA dehydrogenase deficiency (VLCADD). *Journal of inherited metabolic disease, 41(6), 1169-1178.*
3. Ma, Y. et al. (2021). Long chain fatty acyl-CoA synthetase 1 promotes prostate cancer progression by elevation of lipogenesis and fatty acid beta-oxidation. *Oncogene, 40*(10), 1806.