BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Fatty Acid Oxidation (FAO) Assay Kit (Cat #: E-141)

COMPONENTS: FAO Assay Solution- 5 ml (100 wells), **store in aliquots at -80°C after first thawing**
20x Octanoyl-CoA- 0.25 ml, store at -80°C
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: The fatty acid β-oxidation activity assay is based on the oxidation of octanoyl-CoA, which is coupled to NADH-dependent reduction of INT to INT-formazan. The formazan product exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$), allowing for sensitive measurement of FAO activity present in cell/tissue extracts. Reagents are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Wash 10^5 – 10^6 cells twice with ice-cold phosphate-buffered saline (PBS), and remove PBS completely from the cell pellet. Cell pellet should be stored at -80ºC. Tissue sample should be washed with PBS thoroughly to remove blood cells, which can cause inconsistent assay result.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. If lysate is viscous, add more 1x Cell Lysis Solution and repeat pipetting. Centrifuge lysate in a refrigerated microfuge for 3 min at maximal speed. Recover supernatant for assay. Tissue is homogenized in 1x Cell Lysis Solution, and lysate is clarified by centrifugation. Use ~25 mg of tissue per 0.5 ml of 1x Cell Lysis Solution for tissue homogenization.
3. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution to 0.5 – 1 mg/ml. Keep protein sample on ice at all times. Freeze-thawed crude protein lysate can exhibit reduced enzyme activity. Lysate should be stored at -80ºC. Note: Do not use a buffer containing reducing agents or SDS.

Reagent thawing:

Keep thawed FAO Assay Solution and 20x FAO Substrate on ice. Gently agitate solution prior to pipetting. It is important to minimize the time the reagents are thawed. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 20 parts of FAO Assay Solution, e.g. 25 µl dH_2O mixed with 500 µl FAO Assay Solution. Keep freshly prepared control solution on ice during assay.

Reaction solution is prepared by mixing 1 part of 20x FAO substrate and 20 parts of FAO Assay Solution, e.g. 25 µl 20x FAO Substrate mixed with 500 µl FAO Assay Solution. Keep solution on ice and use immediately.

Each protein sample is treated with 50 µl control solution and 50 µl reaction solution in two separate sets.

Enzyme assay:

1. Add 10 µl of each protein sample to a 96-well plate in duplicate. Note: For drug discovery application, add 1 µl of a drug inhibitor to both wells, and mix with sample by pipetting up and down.
2. After all samples have been pipetted to the plate, swiftly add 50 µl control solution to one set of wells and 50 µl reaction solution to another set of wells. Mix contents by gentle agitation for 30 sec. Cover plate and incubate in a humidified 37°C incubator for 1 hr. FAO activity generates cherry red color in wells. Do not use CO_2 incubator.
   Note: Reduce reaction time if red color generated is too dark. Increase sample protein concentration or incubation time if FAO activity is too low.
3. Stop assay by adding 50 µl 3% Acetic acid (not included in the kit) to each control solution well and reaction solution well followed by brief gentle agitation. Measure $O.D._{492\ nm}$ using a plate reader.
4. Subtract control well reading from reaction well reading for each sample. The subtracted O.D. reading is proportional to fatty acid oxidation activity of the sample.

Additional information:

- A 3% Acetic acid solution needs to be prepared for reaction termination. The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.