BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: FAO Assay Solution- 5 ml (for 100 wells), store at -80°C **(shield solution from light during assay)**
25x FAO Substrate (S)- 0.2 ml, store at -80°C **(avoid multiple freeze-thaw cycles)**
10x Sample Buffer- 25 ml, store at 4°C

PRODUCT DESCRIPTION: The FAO assay is based on oxidation of butyryl-CoA coupled to $FADH_2$/NADH-dependent reduction of iodonitrotetrazolium to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$). The kit is for measurement of FAO activity in cell/tissue lysates. Reagents are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. PBS-washed cell/tissue samples should be frozen at -80ºC prior to homogenization. At least ~10^5 cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction.
2. Prepare 1x Sample Buffer by diluting 10x Sample Buffer with dH_2O. Bring up freeze-thawed cells in 50 – 100 µl ice-cold 1x Sample Buffer by pipetting up and down. Adherent cells after deep freezing can be directly harvested from plate by scraping. Leave lysate on ice for 5 min with agitation. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Sample Buffer (~2 mg tissue in 100 µl).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
4. Perform protein assay to determine lysate (supernatant) protein concentration. A suggested sample protein concentration range is 0.5 – 4 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Keep thawed FAO Assay Solution and 25x FAO Substrate (S) on ice and shielded from light. *Do not over thaw.* Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 25 parts of FAO Assay Solution, e.g. 20 µl dH_2O + 500 µl FAO Assay Solution.

Reaction solution is prepared by mixing 1 part of 25x FAO Substrate (S) and 25 parts of FAO Assay Solution, e.g. 20 µl 25x FAO Substrate (S) + 500 µl FAO Assay Solution.

Enzyme assay:

1. Thaw lysates quickly and keep on ice. *Do not over thaw.* Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate. For drug discovery application, mix 1 µl of an inhibitor with sample in wells first.
2. Add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C humidified incubator for 60-120 min. *Do not use CO_2 incubator.* Cherry red color should gradually appear in wells.
3. Remove plate from incubator. Eliminate air bubbles from wells if necessary. Measure absorbance (O.D.) at 492 nm using a plate reader.
4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation. If incubation for 60 min, sample FAO activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.10**. If incubation for 120 min, FAO activity = **ΔO.D. x 4.05**. Enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101) or protein concentration.

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.