

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: FAO Assay Solution- 5 ml (for 100 wells), store at -80°C (**shield solution from light during assay**)
10x FAO Substrate (L)- 0.5 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 palmitoyl-CoA coupled to FADH₂/NADH-dependent reduction of iodinitrotriazolium (INT) to formazan, which exhibits an absorption max at 492 nm ($\epsilon=18 \text{ mM}^{-1}\text{cm}^{-1}$). The kit is for assay 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 -80°C prior to homogenization. At least $\sim 10^5$ 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 dH₂O. 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 ($\sim 2 \text{ mg}$ tissue in 100 μl).
3. Centrifuge lysate in a cold microfuge at $\sim 14,000 \text{ rpm}$ for 5 min. Supernatant is harvested and stored at -80°C.
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 10x FAO Substrate (L) 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 dH₂O and 10 parts of FAO Assay Solution, e.g. 50 μl dH₂O + 500 μl FAO Assay Solution.

Reaction solution is prepared by mixing 1 part of 10x FAO Substrate (L) (vortex vial prior to pipetting) and 10 parts of FAO Assay Solution, e.g., 50 μl 10x FAO Substrate (L) + 500 μl FAO Assay Solution. Gently vortex reaction solution for 10-15 sec to dissolve palmitoyl-CoA.

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 30-60 min. *Do not use CO₂ 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 ($\Delta\text{O.D.}$) for enzyme activity calculation. If incubation for 30 min, sample FAO activity in $\text{IU/L} = \mu\text{mol}/(\text{L}\cdot\text{min}) = \Delta\text{O.D.} \times 1000 \times 70 \mu\text{l} / (30 \text{ min} \times 0.4 \text{ cm} \times 18 \times 20 \mu\text{l}) = \Delta\text{O.D.} \times 16.2$. If incubation for 60 min, FAO activity = $\Delta\text{O.D.} \times 8.1$. Enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101) or protein concentration.

Additional information:

- The 10x FAO(L) substrate may be diluted 20x if precipitates appear in wells during prolonged incubation.
- The assay solution contains dimethyl sulfoxide (DMSO) and iodinitrotriazolium (INT). Please refer to the product webpage or contact us for material SDS information.