TECHNICAL MANUAL

Fatty Acid Oxidation (FAO) Assay Kit

- **SKU CODE:** BR00001
- **SIZE:** 100T
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

Revised: 09/2025

Fatty Acid Oxidation (FAO) Assay Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Application:

Quantitative measurement of fatty acid oxidation

Measuring instrument:

Microplate reader (filters λ=492 nm)

Sample type:

Quantitative measurement of fatty acid oxidation in cells and tissues

2. Storage & Expiry

This product should be stored at -20°C prior to reconstitution. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Assay Genie Fatty Acid Oxidation (FAO) Assay Kit provides a robust, non-radioactive method for quantifying fatty acid β-oxidation activity in cell and tissue lysates.

Fatty acids are a critical energy source, yielding significantly more ATP per carbon than carbohydrates like glucose. In energy-demanding tissues such as the heart, 50–70% of total energy production is derived from fatty acid β-oxidation, which converts fatty acids to acetyl-CoA while generating $FADH_2$ and NADH within the mitochondria. Dysregulation of this pathway is linked to numerous metabolic and genetic disorders, including fatty acid oxidation disorders (FAODs), and its measurement offers valuable insights into disease mechanisms and energy metabolism.

The assay is based on the oxidation of octanoyl-CoA, with the resulting NADH/$FADH_2$ coupled to the reduction of the tetrazolium salt INT into a red-colored formazan product (λmax = 492 nm, ε = 18 $mM^{-1} \cdot cm^{-1}$). The intensity of formazan formation directly correlates with FAO activity, allowing accurate and sensitive detection. This kit is designed for researchers investigating mitochondrial function, metabolic regulation, and disease models where fatty acid metabolism plays a key role.

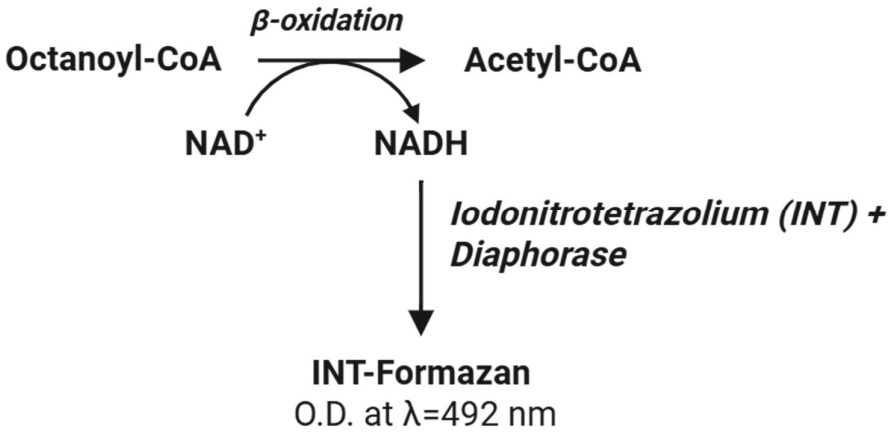


Figure 1: Reaction mechanism of Fatty Acid Oxidation Assay.

4. Kit Contents

No	Component Name	Size (100T)	Storage
1	FAO Assay Solution	5 mL	Store at −20 °C before opening and at −80 °C after reconstitution (protect the solution from light during the assay)
2	20x FAO Substrate (20x Octanoyl-CoA)	0.25 mL	Store at -20°C before opening and at -80°C after reconstitution
3	10x Cell Lysis Solution	25 mL	Store at -20°C
4	Substrate Buffer	5 mL	Store at -20°C
5	Reagent 1 (brown microtube)	0.5 mL	Store at -20°C
6	Reagent 2 (clear microtube)	Yellow powder	Store at -20°C
7	Technical Manual	1 copy	-

Note: *All reagents must be stored according to the specified conditions listed in the table above. The FAO Assay kit is shipped in multiple separate components (in 6 vials) to ensure reagent integrity for at least one week in the absence of dry ice. Store the kit at -20 ℃ prior to reconstitution. Please follow instructions below to reconstitute FAO Assay Solution and 20x FAO Substrate Solution.*

Additional materials required:

1. 37°C incubator.
2. Microplate Reader (filters λ=492 nm)
3. Precision pipettes and disposable pipette tips. Distilled water.
4. Disposable tubes for sample dilution.
5. Absorbent paper

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
5. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

A. Reconstitution of FAO Assay Solution (5 ml)

1. Thaw Reagent 1 (0.5 ml in brown microtube) at room temperature. Keep FAO Assay Solution (5 ml in green/blue cap vial) on ice.
2. Transfer Reagent 1 solution to FAO Assay Solution and immediately mix solution to prevent reagent precipitation. Keep the mixed solution on ice shielded from light.
3. Tap the Reagent 2 vial (clear microtube) to ensure all contents settle at the bottom. Transfer the entire contents of Reagent 2 into the FAO Assay Solution and mix gently to dissolve. If necessary, rinse the clear vial with a small volume of the FAO Assay Solution to ensure complete transfer. Gently agitate FAO Assay Solution on ice shielded from light for ~ 5 min. Reconstituted FAO Assay Solution should appear

bright yellowish. Store FAO Assay Solution at -80℃. FAO Assay Solution should be kept on ice and shielded from light during assay. Avoid multiple freeze-thaw cycles.

B. Reconstitution of 20X FAO Substrate (0.25 mL)

1. Tap 20x FAO Substrate vial (clear microtube) to deposit contents inside.
2. Add 0.25 ml Substrate Buffer (in clear microtube) to 20x FAO Substrate vial and vortex tube to dissolve content. The reconstituted 20x FAO Substrate solution should be stored at -80℃.

7. Sample & Solution preparation

A. Preparation of cell/tissue extracts

1. PBS-washed cell/tissue samples should be frozen at -80℃ prior to homogenization. At least ~10^6 harvested cells or 2 mg of tissue should be used for lysate preparation. Freeze thawing, mechanical grinding, or sonication facilitates protein extraction
2. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH2O. Bring up at least ~106 freeze-thawed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Freeze-thawed tissue is homogenized in ice-cold 1x Cell Lysis Solution (10-20 mg tissue in 0.5 ml).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
4. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 2 mg/ml. Keep lysates on ice during assay.

B. Solution Preparation

1. **Control solution:** Control solution is prepared by mixing 1 part of dH2O and 19 parts of FAO Assay Solution, e.g. 25 µl dH2O mixed with 475 µl FAO Assay Solution. Keep the solution on ice.
2. **Reaction solution:** Reaction solution is prepared by mixing 1 part of 20x FAO Substrate and 19 parts of FAO Assay Solution, e.g. 25 µl 20x FAO Substrate mixed with 475 µl FAO Assay Solution. Keep solution on ice and use immediately.

Note: *Keep thawed FAO Assay Solution and 20x FAO Substrate on ice shielded from light. Do not over thaw. Gently agitate solution prior to pipetting. It is important to minimize the time the reagents are thawed. Freeze solutions immediately after use.*

8. Assay Procedure

1. **Sample Addition:** Thaw the lysates quickly and keep on ice (do not over thaw). Each sample is treated with 50 µl control solution and 50 µl reaction solution. Add 20 µl of each sample to a plain (uncoated) 96-well plate placed on ice duplicate. ***Note:*** *For drug discovery application, add 1 µl of a drug inhibitor to both control and reaction wells and mix by pipetting up and down.*
2. **Reaction Setup:** After all samples have been pipetted to the plate in duplicate, swiftly add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 - 120 min (do not use CO2 incubator). Cherry red color should gradually appear in wells.
3. **Measurement:** Measure O.D.492 nm using a plate reader at 60 min and at 120 min.
4. **Positive control (optional):** If you would like to run a Positive Controls cell with high mitochondrial content should be used e.g. heart or muscle tissue extract (not included).

***Additional information**: The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for SDS information.*

9. Data Analysis

Subtract the absorbance reading of the control well from that of the reaction well for each sample and each time point. Use the resulting value (ΔO.D.) to calculate enzyme activity.

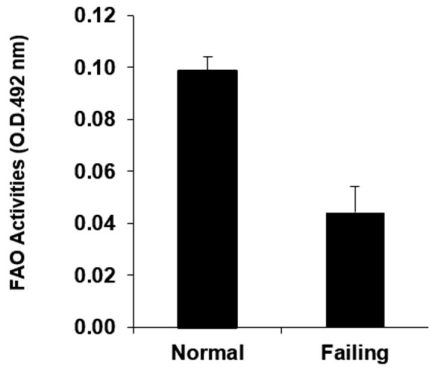
If incubation is 60 min calculate as follows:

FAO activity in IU/L = μmol/(L · min) = ΔO.D. x 1000 × 70μl / (60 min x 0.4 cm x 18 × 20μl) = ΔO.D. x 8.10.

If incubation is120 min calculate as follows:

FAO activity = ΔO.D. x 4.05. Enzyme activity can be presented as units/μg proteins.

10. Typical Data



Tissue homogenates were prepared from normal hamster heart (F1B strain) and failing heart (TO2 strain). FAO activities were assayed by the FAO Assay kit, showing that the failing hamster heart exhibits reduced capacity for fatty acid oxidation.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.