Fatty Acid Oxidation (FAO) Assay Kit

Catalog No: BGT-KET-03060

Size: 96 assays

Intended use

This assay kit can be used to measure fatty acid oxidation (FAO) ability in mammalian cells and tissue samples

Background

Fatty acids (FAs) are basic building blocks for wide variety of lipids, essential components of cells, and are one of primary sources of energy. The major pathway for the degradation of FAs is mitochondrial FA beta-oxidation (FAO). FAO is a key metabolic pathway for energy homeostasis in organs such as the liver, heart and skeletal muscle. FAO is a complicated biochemical event containing many types of enzymes. First, FAs are converted to acyl-CoA form by acyl-CoA synthetase family. Second, acyl-CoA forms are incorporated into mitochondria via the carnitine shuttle pathway. Once acyl-CoA entering to mitochondrial matrix, acyl-CoA (C_n) is converted to acyl-CoA (C_{n-2}) and acetyl-CoA by four stepwise reactions. 1) Dehydrogeneration: Acyl-CoA is oxidized to enoyl-CoA by acyl-CoA dehydrogenases, 2) Hydration: Enoyl-CoA is hydrated to 3-hydroxyacyl-CoA by crotonase, 3) Oxidation: 3-hydroxyacyl-CoA to 3-ketoacyl-CoA, 4) Thiolysis: 3-ketoacyl-CoA to acyl-CoA (C_{n-2}) and acetyl-CoA. Acetyl-CoA is further converted to ATP. The resulting acyl-CoA (C_{n-2}) enters another cycle of FAO to further produce acyl-CoA (C_{n-4}).

Test principle

During the FAO process, substrates and NAD+ are consumed. The resulting NADH is converted into an orange-red substance in the presence of an electron-coupling agent and a chromogen agent, which can be measured at 450 nm.

Kit components & Storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	40 mL × 1 vial	-20°C away from light, up to 6 months
Reagent 2	Co-factor	Powder ×2 vials	
Reagent 3	Substrate	0.22 mL ×1 vial	
Reagent 4	Chromogenic Agent	1.2 mL ×2 vials	
Reagent 5	Standard	Powder ×2 vials	
	Microplate	96 wells	
	Plate Sealer	2 pieces	

Note: Store all reagents strictly as per the conditions in the above table. Do not mix reagents from different kits. For low-volume reagents, centrifuge prior to use to ensure sufficient volume is available.

Materials required but not supplied

Instruments : Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator (37°C)

Reagents: Normal saline (0.9% NaCl)

Reagent preparation

1. Bring all reagents to room temperature (20-25℃) before use.
2. Preparation of **Co-factor working solution :** Dissolve one vial of Co-factor with 1 mL of buffer solution , mix well to dissolve . Keep it on ice away from light before use. Unused working solution can be stored at -20°C away from light and is valid for 3 days.
3. Preparation of **substrate working solution :** Mix buffer solution and substrate in a volume ratio of 4:1. Prepare as needed, keep it on ice away from light before use. Unused working solution should be stored at -20°C away from light and is valid for 3 days. For example, 50 μL of substrate working solution (40 μL of buffer solution +10 μL of substrate).

4. Preparation of **reaction working solution** : For each well, prepare 154 μL of reaction working solution (mix well 144 μL of buffer solution and 10 μL of Co- factor working solution). The reaction working solution should be prepared and used immediately . Keep it on ice away from light before use. Valid for use on the same day.
5. Preparation of **0.5 mmol/L standard solution** : Dissolve one vial of standard with 5 mL of double distilled water, mix well to dissolve . Keep it on ice away from light before use. Unused standard solution can be stored at -20°C away from light and is valid for 3 days.
6. The preparation of standard curve : Dilute 0.5 mmol /L standard solution with double distilled water to a serial concentration .The diluted standard solution should be used up on the same day. The recommended dilution gradient is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**	**0.45**	**0.5**
0.5 mmol/L standard (μL)	0	40	60	80	120	160	180	200
Double distilled water (μL)	200	160	140	120	80	40	20	0

Sample collection

Tissue homogenates:

1. Harvest the amount of tissue needed for each assay (initial recommendation 30 mg).
2. Wash tissue with normal saline.
3. Homgenize 30 mg tissue in 270 μL normal saline with a dounce homogenizer at 4℃.
4. Centrifuge at 10 ,000 ×g for 15 min at 4℃ to remove insolube material . Collect supernatant and keep it on ice before use . It is advisable to test the prepared tissue supernatant within 4 h.
5. Meanwhile, determine the protein concentraion of supernatant.

Cell lysates:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).
2. Wash cells with normal saline.
3. Homogenize 1×10^6 cells in 200 μL normal saline with a ultrasonic cell disruptor at 4℃.
4. Centrifuge at 10 ,000 ×g for 15 min at 4℃ to remove insoluble material . Collect supernatant and keep it on ice before use . It is advisable to test the prepared ecll supernatant within 4 h.
5. Meanwhile, determine the protein concentraion of supernatant.

Sample dilution: The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	2-5
10% Mouse brain tissue homogenate	-
10% Mouse lung tissue homogenate	-
10% Mouse spleen tissue homogenate	-
10% Mouse kidney tissue homogenate	2-5
10% Mouse heart tissue homogenate	2-3
10% Mouse muscle tissue homogenate	1-3
1×10^6 Molt-4 cells	-
1×10^6 HL-60 cells	-
1×10^6 Jurkat cells	-

Note: The diluent is normal saline (0.9% NaCl).

Key point

If the OD value of the sample is greater than 1.5, dilute the sample.

Assay procedure

1. Standard well: Add 50 µL of standard solution with different concentrations to the corresponding wells.
   Sample well: Add 50 µL of sample to the corresponding wells.
   Control well: Add 50 µL of sample to the corresponding wells.
2. Add 165 µL of buffer solution to standard wells, add 20 µL of substrate working solution to sample wells and add 20 µL of buffer solution to control wells.
3. Add 145 µL of reaction working solution to sample and control wells.
4. Add 20 µL of chromogenic agent to each well.
5. Gently shake the microplate for 5 s and incubate at 37℃ for 30 min. Measure the OD value of each well at 450 nm with a microplate reader.

	Standard Well	Sample Well	Control Well
standard solutions with different concentrations(μl)	50	-	-
Sample(μl)	-	50	50
Reagent 1(μl) - buffer solution	165	-	20
Substrate Working solution(μl)	-	20	-
Reaction Working solution(μl)	-	145	145
Reagent 4(μl) - chromogenic agent	20	20	20
Shake the plate for 5 seconds. Incubate at 37°C for 30 minutes. Measure the OD values of each well at 450 nm using a microplate reader.			

Calculation of results

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard# ①) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b).

Tissue and cell samples:

Definition : The amount of enzyme in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol NADH at 37°C is defined as 1 unit.

$$\textbf{FAO ability(U/gprot)} = (\Delta A450 - b) \div a \div T \times 1000 \div C_{pr} \times f$$

ΔA450: OD Sample – OD Control.
T: Reaction time, 30 min.
1000 : 1 mmol/L = 1000 μmol/L.
C_{pr}**:** The concentration of protein in sample, gprot/L.
f: Dilution factor of sample before test.

Appendix I Performance Characteristics

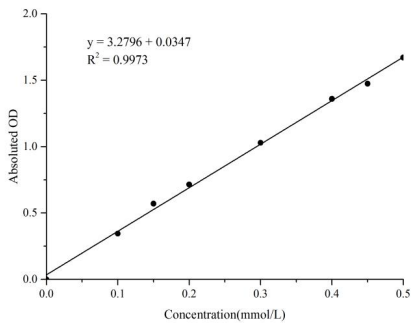
1. Parameter:

Detection Range	0.11-8.33 U/L	Intra-assay Precision	8.2-9.4%
Sensitivity	0.11 U/L	Inter-assay Precision	4.1-5.0%
Dilution Recovery	100-110%		

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator , pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only :

Concentration (mmol/L)	0	0.1	0.15	0.2	0.3	0.4	0.45	0.5
OD	0.05	0.425	0.615	0.763	1.003	1.41	1.534	1.716
	0.05	0.365	0.626	0.766	1.154	1.41	1.513	1.726
Average OD	0.050	0.395	0.621	0.765	1.079	1.410	1.524	1.721
Absoluted OD	0.000	0.345	0.571	0.715	1.029	1.360	1.474	1.671



Appendix II Example Analysis

Example analysis :

Take 50 μL of 10% mouse muscle tissue homogenate, and carry the assay according to the operation steps. The results are as follows: standard curve: y = 3.2796 x+0.0347, the average OD value of the sample well is 1.860, the average OD value of the control well is 1.600, ΔA450 = 1.860 - 1.600 = 0.260, the concentration of protein is 5.201 gprot/L, and the calculation result is:

FAO ability(U/grpot) = (0.260 - 0.0347) ÷ 3.2796 ÷ 30 × 1000 ÷ 5.201 = 0.44 U/gprot

Detect 10% mouse muscle tissue homogenate (the concentration of protein is 5.201 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 3.931 gprot/L), 10% mouse spleen tissue homogenate (the concentration of protein is 8.297 gprot/L), 1×10^6 Molt-4 cells homogenate (the concentration of protein is 0.165 gprot/L), according to the protocol, the result is as follows :